PGPUB-DOCUMENT-NUMBER: 20030199048

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030199048 A1

TITLE: Stereoselective estarase from aspergillus oryzae

PUBLICATION-DATE: October 23, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Ostergaard, Peter Rahbek	Virum		DK	
Hjort, Carsten M	Vaerlose		DK	
Deussen, Heinz-Josef	Soborg		DK	
Zundel, Magali	Soborg		DK	
Ebdrup, Soren	Roskilde		DK	
Christensen, Soren	Jyllinge		DK	
Patkar, Shamkant Anant	Lyngby		DK	

APPL-NO: 10/ 343879

DATE FILED: February 5, 2003

PCT-DATA:
APPL-NO: PCT/DK01/00508
DATE-FILED: Jul 19, 2001
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/136, 435/135 , 435/196 , 435/252.33 , 435/254.3 , 435/320.1 , 435/69.1 , 536/23.2

ABSTRACT:

An esterase isolated from Aspergillus oryzae is capable of stereoselective hydrolysis of chiral esters and also has arylesterase activity (EC 3.1.1.2) and **feruloyl esterase** activity (EC 3.1.1.73). The esterase has only a limited homology to known amino acid sequences.

---------- KWIC ---------

Abstract Paragraph - ABTX (1):

An esterase isolated from Aspergillus oryzae is capable of stereoselective hydrolysis of chiral esters and also has arylesterase activity (EC 3.1.1.2) and **feruloyl esterase** activity (EC 3.1.1.73). The esterase has only a limited

homology to known amino acid sequences.

Summary of Invention Paragraph - BSTX (5):

[0003] Enzymes with **ferulic acid esterase** activity are known, e.g., from Aspergillus oryzae. M. Tenkanen, Biotechnology and Applied Biochemistry, 27 (1), 19-24 (1998)); M. Tenkanen et al., J. Biotechnol., 18 (1-2), 69-84 (1991).

Summary of Invention Paragraph - BSTX (8):

[0005] The inventors have isolated an esterase from Aspergillus oryzae which is capable of stereoselective hydrolysis of chiral esters and also has arylesterase activity (EC 3.1.1.2) and **feruloyl esterase** activity (EC 3.1.1.73). The novel esterase has only a limited homology to known amino acid sequences. The inventors also isolated a gene encoding the novel esterase and cloned it into an E. coli strain.

Detail Description Paragraph - DETX (13):

[0033] The esterase also has arylesterase activity (EC 3.1.1.2) and **feruloyl esterase** activity (EC 3.1.1.73) and is useful in hydrolyzing feruloyl esters into ferulic acid and alcohol. It is useful for the release of ferulic acid bound to hemicellulose in the degradation of plant material and plant cell walls, e.g. as described in GB 2301103 and WO 200014234. It may also be used for the production of vanillic acid in analogy with U.S. Pat. No. 5,955,137.

PGPUB-DOCUMENT-NUMBER: 20030175968

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030175968 A1

TITLE: Gene targeting method

PUBLICATION-DATE: September 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Golic, Kent G.	Salt Lake City	UT	US	
Rong, Yikang S.	Salt Lake City	UT	US	
Drews, Gary N.	Salt Lake City	UT	US	

APPL-NO: 10/ 204039

DATE FILED: October 30, 2002

PCT-DATA:
APPL-NO: PCT/US01/07051
DATE-FILED: Mar 1, 2001
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/455, 435/468

ABSTRACT:

The invention related to a method of gene targeting in a transformable host organism, and compositions useful for carrying out the method. The method of gene targeting provides improvement over previous gene targeting methods since it is generally applicable over a wide variety of transformable organisms. It provides time savings in producing organisms with specific gene modifications, and it does not require a pluripotential cell line. The targeting method of the invention exploits the endogenous cellular process of homologous recombination to implement gene targeting at essentially any known gene.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority from U.S. Provisional Patent Application Nos. 60/258,682 filed Dec. 28, 2000, 60/188,672, filed Mar. 13, 2000, and 60/187,220, filed Mar. 3, 2000.

---------- KWIC ---------

Detail Description Paragraph - DETX (64):

[0105] Techniques for genetically engineering plant cells and/or tissue with an expression cassette comprising an inducible promoter or chimeric promoter fused to a heterologous coding sequence and a transcription termination sequence are to be introduced into the plant cell or tissue by Agrobacterium-mediated transformation, electroporation, microinjection, particle bombardment or other techniques known to the art. The expression cassette advantageously further contains a marker allowing selection of the heterologous DNA in the plant cell, e.g., a gene carrying resistance to an antibiotic such as kanamycin, hygromycin, gentamycin, or bleomycin. Assays for **phenolic acid esterase** and/or xylanase enzyme production are taught herein or in U.S. Pat. No. 5,824,533, for example, and other assays are available to the art.

PGPUB-DOCUMENT-NUMBER: 20030175940

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030175940 A1

TITLE: Cell-wall degrading enzyme variants

PUBLICATION-DATE: September 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Schroder Glad, Sanne O.	Ballerup		DK	
Andersen, Carsten	Vaerlose		DK	
Schulein, Martin	Copenhagen		DK	
Dela, Hanne	Copenhagen		DK	
Peter, Torben	Frandsen		DK	

APPL-NO: 10/ 403192

DATE FILED: March 31, 2003

RELATED-US-APPL-DATA:

child 10403192 A1 20030331

parent division-of 09910505 20010719 US PENDING

non-provisional-of-provisional 60290724 20010514 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	PA 2000 01117	2000DK-PA 2000 01117	July 19, 2000
DK	PA 2001 00705	2001DK-PA 2001 00705	May 4, 2001
DK	PA 2001 00734	2001DK-PA 2001 00734	May 10, 2001

US-CL-CURRENT: 435/232, 435/252.3 , 435/264 , 435/320.1 , 435/69.1 , 510/320 , 536/23.2 , 702/19

ABSTRACT:

The present invention relates to variants of a cell-wall degrading enzyme having a beta-helix structure, which variant has at least one substituent in a position determined by identifying all residues potentially belonging to a stack; characterizing the stack as interior or exterior; characterizing the stack as polar, hydrophobic or aromatic/heteroaromatic based on the dominating characteristics of the parent or wild-type enzyme stack residues and/or its orientation relative to the beta-helix (interior or exterior); optimizing all stack positions of a stack either to hydrophobic aliphatic amino acids, hydrophobic aromatic or polar amino acids by allowing mutations within one or all positions to amino acids belonging to one of these groups; measuring

thermostability of the variants by DSC or an application-related assay such as a Pad-Steam application test; and selecting the stabilized variants. Variant of a wild-type parent pectate lyase (EC 4.2.2.2) having the conserved amino acid residues D111, D141 or E141, D145, K165, R194 and R199 when aligned with the pectate lyase comprising the amino acid sequence of SEQ ID NO: 2 are preferred.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a divisional of application Ser. No. 09/910,505 filed Jul. 19, 2001, which claims priority or the benefit under 35 U.S.C. 119 of U.S. Provisional Application No. 60/290,724 filed May 14, 2001, and Danish Application Nos. PA 2000 01117, PA 2001 00705 and PA 2001 00734 filed Jul. 19, 2000, May 4, 2001 and May 10, 2001, respectively, the contents of which are fully incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (45):

[0045] Endo- and exo-xylanases and accessory enzymes such as glucuronidases, arabinofuranosidases, acetyl xylan **esterase and ferulic** acid or **coumaric acid esterase** have been summarized by Kormelink (1992, Ph.D.-thesis, University of Wageningen, The Netherlands). They are produced by a wide variety of microorganisms and have varying temperature and pH optima.

PGPUB-DOCUMENT-NUMBER: 20030175893

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030175893 A1

TITLE: Novel method to isolate mutants and to clone the complementing gene

PUBLICATION-DATE: September 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
De Graaff, Leendert Hendrik	Oosterbeek		NL	
Van Den Broeck, Henrietta Catharina	Bennekom		NL	
Visser, Jacob	Wageningen		NL	

APPL-NO: 10/ 419969

DATE FILED: April 22, 2003

RELATED-US-APPL-DATA:

child 10419969 A1 20030422

parent division-of 08981729 19971223 US GRANTED

parent-patent 6177261 US

child 08981729 19971223 US

parent a-371-of-international PCT/NL96/00259 19960624 WO UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
EP	95201707.7	1995EP-95201707.7	June 23, 1995
EP	95202346.3	1995EP-95202346.3	August 30, 1995

US-CL-CURRENT: 435/69.1, 435/254.3 , 435/320.1 , 530/350 , 536/23.5

ABSTRACT:

The subject invention lies in the field of microorganism mutation and selection of the mutants. In particular the invention is directed at obtaining metabolic mutants in a simple, direct and specific manner. In a preferred embodiment it is also possible to obtain desired mutants not comprising recombinant DNA, thereby facilitating incorporation thereof in products for human consumption or application, due to shorter legislative procedures. The method according to the invention involves random mutation and specific selection of the desired metabolic mutant.

A nucleic acid cassette comprising a nucleic acid sequence encoding a bidirectional marker, said nucleic acid cassette further comprising a basic transcriptional unit operatively linked to the nucleic acid sequence encoding the bidirectional marker and said nucleic acid cassette further comprising an inducible enhancer or activator sequence linked to the basic transcription unit in such a manner that upon induction of the enhancer or activator sequence the bidirectional marker encoding nucleic acid sequence is expressed, said inducible enhancer or activator sequence being derived from a gene associated with activity of part of metabolism, said inducible enhancer or activator sequence being derived from a gene associated with metabolism is claimed as is application thereof in a selection method for mutants.
In addition a regulator gene xlnR encoding an activating regulator of an inducible enhancer or activator sequence and application of said gene and/or its expression product in overexpression of homologous or heterologous protein or peptide is described. Knockout mutants wherein said gene is absent or inactivated and mutants with increased or decreased DNA binding capacity are also claimed.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (108):

[0106] Genes of particular interest for expressing using the expression cassette according to the invention or in combination with a nucleic acid sequence according to the invention are those encoding enzymes. Suitable genes for expressing are genes encoding xylanases, glucanases, oxidoreductases such as hexose oxidase, .alpha.-glucuronidase, lipase, **esterase, ferulic acid esterase** and proteases. These are non limiting examples of desirable expression products. A number of sequences are known in the state of the art comprising the genes mentioned and such information is readily available to the person skilled in the art and is to be considered incorporated herein. The genes can either be readily, synthesized on the basis of known sequences in the literature or databases or be derived from organisms or vectors comprising--hem in a standard manner known per se and are considered to be knowledge readily available to the person skilled in the art not requiring further elucidation.

Claims Text - CLTX (27):

27. A combination nucleic acid cassette according to any of claims 20-26, wherein the homologous or heterologous sequence encodes a xylanase, glucanase, .alpha.-glucuronidase, lipase, **esterase, ferulic acid esterase,** a protease or an oxidoreductase such as hexose oxidase.

PGPUB-DOCUMENT-NUMBER: 20030175384

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030175384 A1

TITLE: Method for the extraction of aleurone from bran

PUBLICATION-DATE: September 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bohm, Arturo	Oberuzwil		CH	
Bogoni, Carlo	Winterthur		CH	
Behrens, Raimund	Konstanz		DE	
Otto, Thomas	Konstanz		DE	

APPL-NO: 10/ 344809

DATE FILED: February 14, 2003

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DE	100 41 156.8	2000DE-100 41 156.8	August 21, 2000
DE	100 42 188.1	2000DE-100 42 188.1	August 28, 2000

PCT-DATA:
APPL-NO: PCT/CH01/00506
DATE-FILED: Aug 20, 2001
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 426/52

ABSTRACT:

The invention relates to a method for the preparation of aleurone from bran, in particular, wheat bran, for the extraction of aleuronic cells in particular from wheat grain, whereby the mainly aleurone-containing aleuronic components are separated from the mainly non-aleurone-containing non-aleuronic components in the bran and the aleurone-containing components are then isolated. The separation can be achieved by biochemical/enzymatic means and/or by mechanical-abrasive means. The subsequent isolation and extraction can be achieved by wet and/or dry separating methods.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (55):

[0054] It is particularly convenient if the biochemical substance contains at least one of the enzymes endoxylanase, beta-xylosidase, arabinofuranosidase, acetylesterase, xyloacetylesterase, and **feruloyl esterase** in this hydrated medium with which the mill product fractions are mixed.

Claims Text - CLTX (59):

59. Method according to claim 57, characterized in that the biochemical substance preferably contains at least one of the enzymes endoxylanase, beta-xylosidase, arabinofuranosidase, acetylesterase, xyloacetylesterase, and **feruloyl esterase** in a hydrated medium with which the mill product fractions are mixed into a hydrated mixture.

PGPUB-DOCUMENT-NUMBER: 20030175383

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030175383 A1

TITLE: Method of improving dough and bread quality

PUBLICATION-DATE: September 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bojsen, Kirsten	Hellerup		DK	
Poulsen, Charlotte Horsmans	Braband		DK	
Soe, Jorn Borch	Tilst		DK	

APPL-NO: 10/ 150429

DATE FILED: May 17, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60347007 20020109 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
GB	0112226.6	2001GB-0112226.6	May 18, 2001

US-CL-CURRENT: 426/20

ABSTRACT:

A method of preparing a flour dough, said method comprising adding to the dough components an enzyme that under dough conditions is capable of hydrolysing a glycolipid and a phospholipid, wherein said enzyme is incapable, or substantially incapable, of hydrolysing a triglyceride and/or a 1-monoglyceride, or a composition comprising said enzyme, and mixing the dough components to obtain the dough.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (104):

[0095] Alternatively, an enzyme which does not, initially at least, have the specific properties as defined herein can be modified, for example by altering the amino acid sequence thereof, in order to provide an enzyme having the properties as defined herein and having the desired substrate specificity. It is known in the art to modify enzymes by random mutagenesis (U.S. Pat. No. 4,814,331, WO 93/01285 and WO 95/22615) and to modify lipolytic enzymes by site-specific mutagenesis (WO 97/04079) to obtain improved performance thereof.

The generally used concept has been to insert, delete or substitute amino acids within the structural part of the amino acid chain of a lipolytic enzyme in question. A suitable enzyme for modification is one that can hydrolyse ester bonds. Such enzymes include, for example, lipases, such as triacylglycerol lipase (E.C. 3.1.1.3), lipoprotein lipase (E.C. 3.1.1.34), monoglyceride lipase (E.C. 3.1.1.23), lysophospholipase, **ferulic acid esterase** and esterase (E.C. 3.1.1.1, E.C. 3.1.1.2) and lipolytic acyl hydrolases (E.C. 3.1.1.26) and phosphatidylinositol deacylase (E.C. 3.1.1.52).

PGPUB-DOCUMENT-NUMBER: 20030167511

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030167511 A1

TITLE: Production of p-hydroxybenzoic acid

PUBLICATION-DATE: September 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Narbad, Arjan	Norfolk		GB	
Rhodes, Michael John Charles	Norfolk		GB	
Gasson, Michael John	Norfolk		GB	
Walton, Nicholas John	Norfolk		GB	

APPL-NO: 10/ 199405

DATE FILED: July 17, 2002

RELATED-US-APPL-DATA:

child 10199405 A1 20020717

parent division-of 09733383 20001207 US PENDING

child 09733383 20001207 US

parent division-of 09155185 19980922 US ABANDONED

child 09155185 19980922 US

parent a-371-of-international PCT/GB97/00809 19970324 WO UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
GB	GB96/06187	1996GB-GB96/06187	March 23, 1996

US-CL-CURRENT: 800/278, 435/136 , 536/23.2

ABSTRACT:

One aspect of the present invention relates to a transgenic plant which, by presence of a transgene, is able to produce p-hydroxybenzoic acid or a .beta.-D-glycoside or .beta.-D-glucose ester thereof. A method is also disclosed for producing p-hydroxybenzoic acid or a .beta.-D-glycoside or .beta.-D-glucose ester thereof using a transgenic plant of the present invention.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (50):

[0050] Trans-ferulic acid or a salt thereof is readily available from plant material Suitably, trans-ferulic acid or a salt thereof is released from the plant material by the action of **ferulic acid esterase**. Thus, in a particularly preferred embodiment of the invention the trans-ferulic acid or salt thereof is provided by the action of **ferulic acid esterase** on plant material.

Summary of Invention Paragraph - BSTX (51):

[0051] Trans-ferulic acid and trans4-coumaric acid can together represent up to 1.5% by weight of the cell walls of temperate grasses (R. D. Hartley and E. C. Jones, Phytochemistry 16, 1531-1534 (1977)). Trans-ferulic acid is reported to comprise 0.5% (w/w) of wheat bran (M. C. Ralet, J. -F. Thibault and G. Della Valle, J. Cereal Sci. 11, 249-259 (1990)), 3.1% of maize bran (L. Saulnier, C. Marot, E. Chanliaud and J. -F. Thibault, Carbohydr. Polym. 26, 279-287 (1995)) and 0.8% of sugar beet pulp (V. Micard, G. M. G. C. Renard and J. -F. Thibault, Lebensm. -Wiss. u-Technol. 27, 59-66 (1994)). These materials are amongst the preferred sources of trans-ferulic acid. Since trans-ferulic acid is present esterified with cell-wall polysaccharides, hydrolysis is essential. Alkaline or acid hydrolysis is possible, but enzymic hydrolysis is preferred. Typically, the initial step is the partial enzymic hydrolysis of carbohydrates (arabinans, xylans, rhamnogalacturanans) to which trans-ferulate is linked, followed by the release of trans-ferulate from the oligosaccharide fragments by trans-**ferulic acid esterase** activity. In practice, both steps may occur simultaneously in the reaction mixture. Descriptions of representative laboratory-scale processes are available in the literature (for example see L. P. Christov and B. A. Prior, Enzyme Microb. Technol. 15, 460-475 (1993)); C. B. Faulds and G. Williamson, Appl. Microbiol. Biotechnol. 43, 1082-1087 (1995); C. B. Faulds, P. A. Kroon, L. Saulnier, J. -F. Thibault and G. Williamson, Carbohydrate Polymers 27, 187-190 (1995)). Phenolic acid-releasing enzymes have been reported from a number of microorganisms, including Streptomyces olivochromogenes (C. B. Faulds and G. Williamson, J. Gen. Microbiol. 137, 2337-2345 (1991)), Penicillium pinophilum (A. Castanares, S. I. McCrae and T. M. Wood, Enyme Microb. Technol. 14, 875-884 (-1992)), Neocallimaslix spp. (W. S. Borneman, R. D. Hartley, W. H. Morrison, D. E. Akin and L. G. Ljungdahl, Appl. Microbiol. Biotechnol. 33, 345-351 (1990)), Schizophyllum commune (R. C. MacKenzie and D. Bilous, Appl. Envir. Microbiol. 54, 1170-1173 (1988)) and Aspergillus spp. (M. Tenkanen, J. Schuseil, J. Puls and K. Poutanen, J. Biotechnol. 18, 69-84 (1991); C. B. Faulds and G. Williamson, Microbiology 140, 779-787 (1994)). A trans-**ferulic acid esterase** (XYLD) has been characterised from Pseudomonas fluorescens subsp. cellulosa, together with an arabinofuranosidase (XYLC) and an endoxylanase (XYLB; L. M. A. Ferreira, T. M. Wood, G. Williamson, C. B. Faulds, G. P. Hazlewood and H. J. Gilbert, Biochem. J. 294, 349-355 (1993)). The genes for all three enzymes have been isolated (G. P. Hazlewood and H. J. Gilbert, in "Xylans and Xylanases", eds. J. Visser, G. Beldman, M. A. Kusters-van Someren and A. G. J. Voragen, Elsevier, Amsterdam, pp 259-273 (1992)). All of these references are incorporated herein by reference.

Summary of Invention Paragraph - BSTX (52):

[0052] Thus, advantageously the trans-ferulic acid or a salt thereof may be provided by the action of trans-**ferulic acid esterase** on said ester. More particularly, it is advantageous to introduce a gene encoding said esterase into a host cell or organism which is being used in the methods of the invention. Thus, it is convenient to introduce a trans-**ferulic acid esterase** gene, such as the aforementioned XYLD gene, into a plant which is being used in the methods of the invention.

PGPUB-DOCUMENT-NUMBER: 20030144165

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030144165 A1

TITLE: Lipolytic enzyme variant

PUBLICATION-DATE: July 31, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Roggen, Erwin Ludo	Lyngby		DK	

APPL-NO: 10/ 258783

DATE FILED: October 28, 2002

PCT-DATA:
APPL-NO: PCT/DK01/00286
DATE-FILED: Apr 30, 2001
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 510/226, 426/20 , 435/198 , 435/254.2 , 435/320.1 , 435/69.1 , 510/320

ABSTRACT:

The properties of a fungal lipolytic enzyme can be altered by substituting amino acid residues corresponding to certain specified amino acid residues in the T. lanuginosus lipase. The altered property may be, e.g., an increased thermostability, an altered pH dependence, or an altered substrate specificity.

---------- KWIC ---------

Detail Description Paragraph - DETX (3):

[0016] The lipolytic enzyme to be used in the present invention is classified in EC 3.1.1 Carboxylic Ester Hydrolases according to Enzyme Nomenclature (available at http://www.chem.qmw.ac.uk/iubmb/enzyme). The substrate specificity may include activities such as EC 3.1.1.3 triacylglycerol lipase, EC 3.1.1.4 phospholipase A2, EC 3.1.1.5 lysophospholipase, EC 3.1.1.26 galactolipase, EC 3.1.1.32 phospholipase A1, EC 3.1.1.73 **feruloyl esterase**.

Detail Description Paragraph - DETX (4):

[0017] The parent lipolytic enzyme is fungal and has an amino acid sequence that can be aligned with SEQ ID NO: 1 which is the amino acid sequence shown in positions 1-269 of SEQ ID NO: 2 of U.S. Pat. No. 5,869,438 for the lipase from Thermomyces lanuginosus (synonym Humicola lanuginosa), described in EP 258 068 and EP 305 216. The parent lipolytic enzyme may particularly have an amino acid sequence with at least 50% homology with SEQ ID NO: 1. In addition to the lipase from T. lanuginosus, other examples are a lipase from Penicillium camembertii (P25234), lipase/phospholipase from Fusarium oxysporum (EP 130064, WO 98/26057), lipase from F. heterosporum (R87979), lysophospholipase from Aspergillus foetidus (W33009), phospholipase A1 from A. oryzae (JP-A 10-155493), lipase from A. oryzae (D85895), lipase/**ferulic acid esterase** from A. niger (Y09330), lipase/**ferulic acid esterase** from A. tubingensis (Y09331), lipase from A. tubingensis (WO 98/45453), lysophospholipase from A. niger (WO 98/31790), lipase from F. solanii having an isoelectric point of 6.9 and an apparent molecular weight of 30 kDa (WO 96/18729).

PGPUB-DOCUMENT-NUMBER: 20030108642

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030108642 A1

TITLE: Penicillium funiculosum strain useful for the production of enzymes

PUBLICATION-DATE: June 12, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sabatier, Alain	Paris		FR	
Fish, Neville Marshall	Stockport		GB	
Haigh, Nigel Paterson	Huddersfield		GB	

APPL-NO: 10/ 299393

DATE FILED: November 19, 2002

RELATED-US-APPL-DATA:

child 10299393 A1 20021119

parent division-of 09462246 20000407 US PENDING

child 09462246 20000407 US

parent a-371-of-international PCT/IB99/00856 19990506 WO UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
EP	EP9840101.5	1998EP-EP9840101.5	May 6, 1998

US-CL-CURRENT: 426/37, 435/196 , 435/200 , 435/254.5

ABSTRACT:

The present invention relates to novel micro-organism, Penicillium funiculosum, to new enzymes mixture obtained from it and nucleic acid sequences thereto.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (47):

[0043] We obtain a new enzymes mixture produced by Penicillium funiculosum. This enzymes mixture contains new enzymes such as cellulases, .beta.-glucanases, xylanases, xylanase accessory enzymes such as arabinofuranosidase and **feruloyl esterases**.

Summary of Invention Paragraph - BSTX (49):
[0045] The enzyme preparations is characterised by assays that include assays for cellulase, cellobiohydrolase, .beta.-glucosidase, endo-1,3(4)-.beta.-glucanase, laminarinase endo-1,4-.beta.-xylanase (using different substrates), .beta.-xylosidase, arabinofuranosidase and **feruloyl esterase** (using different substrates) activities.

Summary of Invention Paragraph - BSTX (103):
[0099] 1.12 **Feruloyl Esterase** by the FAXX Method

Summary of Invention Paragraph - BSTX (104):
[0100] An assay of **feruloyl esterase (ferulic acid esterase)** is based on the enzymatic hydrolysis of O[5-O-(trans-feruloyl)-.alpha.-L-arabinofuran-osyl]-(1.fwdarw.3)-O-.beta.-D-xylopyranosyl-(1.fwdarw.4)-D-xylopyranose (FAXX). FAXX is prepared from enzyme-hydrolysed wheat bran, purified and characterised by NMR. FAXX hydrolysis is measured spectophotometrically.

Summary of Invention Paragraph - BSTX (106):
[0102] One unit of **feruloyl esterase** activity on FAXX is defined as the amount of enzyme which converts 1 .mu.mole substrate to product per minute under the conditions of the assay (37.degree. C. and pH 6.0).

Summary of Invention Paragraph - BSTX (107):
[0103] 1.13 **Feruloyl Esterase** by the Ara.sub.2F Method

Summary of Invention Paragraph - BSTX (108):
[0104] An assay of **feruloyl esterase (ferulic acid esterase)** is based on the enzymatic hydrolysis of Ara.sub.2F (terulic acid linked 1,2 to arabinose). Ara.sub.2F is prepared from enzyme-hydrolysed sugar beet pulp, purified and characterised by NMR. Ara.sub.2F hydrolysis is measured spectophotometrically.

Summary of Invention Paragraph - BSTX (110):
[0106] One unit of **feruloyl esterase** activity on Ara.sub.2F is defined as the amount of enzyme which converts 1 .mu.mole substrate to product per minute under the conditions of the assay (37.degree. C. and pH 6.0).

Summary of Invention Paragraph - BSTX (111):
[0107] 1.14 **Feruloyl Esterase** by the Hydrolysis of Methyl Esters: Methyl Ferulic Acid (MFA); Methyl Caffeic Acid (MCA); Methyl Sinapic Acid (MSA); Methyl p-coumaric Acid (MpCA) Methods

Summary of Invention Paragraph - BSTX (112):

[0108] An assay of **feruloyl esterase (ferulic acid esterase)** is based on the enzymatic hydrolysis of methyl esters of ferulic acid (MFA), caffeic acid (MCA), sinapic acid (MSA) and p-coumaric acid (MpCA). Methyl ester hydrolysis is measured in 0.1M MOPS buffer, pH 6.0 at 37.degree. C. Assays are based on two different techniques.

Summary of Invention Paragraph - BSTX (115):
[0111] One unit of **feruloyl esterase** activity is defined as the amount of enzyme which converts 1 .mu.mole substrate to product per minute under the conditions of the assay (37.degree. C. and pH 6.0).

Summary of Invention Paragraph - BSTX (196):
[0192] 3.3 Properties of **Feruloyl Esterases**

Summary of Invention Paragraph - BSTX (199):
[0195] The enzymes mixture contains at least two distinct **feruloyl esterases**. One of these (FaeB) has a molecular weight of 38,945-41,051 Da by mass spectrometry (35,450 Da from the primary amino acid sequence and 37 kDa by SDS-PAGE). FaeB has a pI of 4.2, it is a type B **feruloyl esterase** and is specific for MpCA and Ara.sub.2F substrates (activity against MpCA, MCA, MFA and Ara.sub.2F; but not against MSA and FAXX).

Summary of Invention Paragraph - BSTX (200):
[0196] The other **feruloyl esterase** (FaeA) has a molecular weight of 29 kDa (by SDS-PAGE). FaeA has a pI of 4.65, it is a type A **feruloyl esterase** and is specific for FAXX and MSA substrates (activity against MSA, MCA, MFA and FAXX but not MPCA Ara.sub.2F).

Summary of Invention Paragraph - BSTX (203):
[0199] The stained IEF gel indicates the presence of very many proteins in cellulase with pI's ranging from very acidic (pI 2.4) to about pI 7. Most of the proteins are acidic (pI range 2.4-5). Two peaks of **feruloyl esterase** activity were detected in fractions cut from the gel. One, corresponding to FaeB, had a pI of 4.2 and activity only against MFA and MpCA (not MSA) . The other, corresponding to FaeA, had a pI of 4.65 and activity against all three substrates tested .

Summary of Invention Paragraph - BSTX (206):
[0202] The enzymes mixture contains at least two distinct **feruloyl esterases**. One corresponding to FaeB (pI 4.2) has a molecular weight of 37 kDa. The other, corresponding to FaeA (pI 4.65) has a molecular weight of 29 kDa.

Summary of Invention Paragraph - BSTX (209):
[0205] Assays for **feruloyl esterase** activity performed on the enzymes

mixture using the spectrophotometric method

Summary of Invention Paragraph - BSTX (210):
[0206] The enzymes mixture contains activity against all the substrates tested. With the methyl esters, activity is highest against MpCA and lowest against MSA. The activities against Ara.sub.2F and FAXX are higher than against the methyl esters which is indicative that the esterase activities are due to true **feruloyl esterases** and not general esterases or side activities of other cell wall-degrading esterases (e.g. acetyl xylan esterase, pectin esterase).

Summary of Invention - Table CWU - BSTL (9):
9TABLE A Relative activities against relevant different substrates Results with Penicillium Methods used in the tests funiculosum Cellulase (DNS CMC method, pH 5.0) [1.1] 3.14 Cellobiohydrolase (p-nitrophenyl .beta.-D-cellobiopyranoside 0.022 method, pH 5.0) [1.2] .beta.-Glucosidase (p-nitrophenyl .beta.-D-glucobiopyranoside method, 0.157 pH 5.0) [1.3] Endo-1,3(4)-.beta.-glucanase (DNS barley .beta.-glucan method, pH 5.0) 7.23 [1.4] Endo-1,3(4)-.beta.-glucanase (azo-barley .beta.-glucan method, pH 4.6) 1+/- [1.5] Laminarinase (DNS laminarin method, pH 5.0) [1.6] 0.30 Endo-1,4-.beta.-xylanase (DNS birchwood xylan method, pH 3.5) 9.16 [1.7] Endo-1,4-.beta.-xylanase (DNS wheat arabinoxylan method, pH 3.5) 8.67 [1.8] Endo 1,4-.beta. xylanase (viscometric wheat arabinoxylan method, 9.80 pH 5.5) [1.9] .beta.-Xylosidase (p-nitrophenyl .beta.-D-xylobiopyranoside method) 0.0047 [1.10] .alpha.-N-Arabinofuranosidase (p-nitrophenyl .alpha.-L-arabinofuranoside 0.0017 method) [1.11] **Feruloyl esterase** (FAXX method) [1.12] 0.000254 Feruloyl esterase (Ara.sub.2F method) [1.13] 0.000349 **Feruloyl esterase** (MFA spectrophotometric method) [1.14] 0.000135 **Feruloyl esterase** (MCA spectrophotometric method) [1.14] 0.000174 **Feruloyl esterase** (MSA spectrophotometric method) [1.14] 0.000049 **Feruloyl esterase** (MpCA spectrophotometric method) [1.14] 0.000216

Claims Text - CLTX (5):
5- New **feruloyl esterases** obtainable from Penicillium funiculosum IMI 378536.

US-PAT-NO: 6664088

DOCUMENT-IDENTIFIER: US 6664088 B2

TITLE: Production of vanillin

DATE-ISSUED: December 16, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Narbad; Arjan	Norfolk	N/A	N/A	GB
Rhodes; Michael John Charles	Norfolk	N/A	N/A	GB
Gasson; Michael John	Norfolk	N/A	N/A	GB
Walton; Nicholas John	Norfolk	N/A	N/A	GB

APPL-NO: 09/733383

DATE FILED: December 7, 2000

PARENT-CASE:

This application is a divisional of U.S. patent application Ser. No. 09/155,183 (now U.S. Pat. No. 6,323,011 B1), which was filed on May 3, 1999 (and accepted May 3, 1999) under 35 U.S.C. .sctn.371 as a national stage application of PCT/GB97/00809 filed Mar. 24, 1997, claiming priority of Great Britain Application No. 9606187.4 filed Mar. 23, 1996.

The biological material listed below has been deposited under the Budapest Treaty at The National Collections of Industrial and Marine Bacteria Limited (23 St. Machar Drive, Aberdeen AB2 1RY, Scotland, UK):

NCIMB No. Description Date of Deposit 40783 Pseudomonas fluorescens biovar V (strain Jan. 15, 1996 AN103) 40777 Escherichia coli (strain pFI793) containing Dec. 15, 1995 cosmid pFI703

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9606187	March 23, 1996

US-CL-CURRENT: 435/195, 435/147 , 435/183 , 435/219 , 435/232 , 435/252.3 , 435/278 , 435/320.1 , 435/874 , 536/23.2

ABSTRACT:

A method of producing vanillin comprising the steps of: (1) providing trans-ferulic acid or a salt thereof; and (2) providing trans-ferulate: CoASH ligase activity (enzyme activity I), trans-feruloyl ScoA hydratase activity (enzyme activity II), and 4-hydroxy-3-methoxyphenyl-.beta.-hydroxy-propionyl SCoA (HMPHP SCoA) cleavage activity (enzyme activity III). Conveniently the enzymes are provided by Pseudomonas fluorescens Fe3 or a mutant or derivative

thereof. Polypeptides with enzymes activities II and III and polynucleotides encoding the polypeptides. Use of the polypeptides or the polynucleotides in a method for producing vanillin is also provided.

16 Claims, 27 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 27

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Brief Summary Text - BSTX (49):

Trans-ferulic acid or a salt thereof is readily available from plant material. Suitably, trans-ferulic acid or a salt thereof is released from the plant material by the action of **ferulic acid esterase**. Thus, in a particularly preferred embodiment of the invention the trans-ferulic acid or salt thereof is provided by the action of **ferulic acid esterase** on plant material.

Brief Summary Text - BSTX (50):

Trans-ferulic acid and trans4-coumaric acid can together represent up to 1.5% by weight of the cell walls of temperate grasses (R. D. Hartley and E. C. Jones, Phytochemistry 16, 1531-1534 (1977)). Trans-ferulic acid is reported to comprise 0.5% (w/w) of wheat bran (M. C. Ralet, J.-F. Thibault and G. Della Valle, J. Cereal Sci. 11, 249-259 (1990)), 3.1% of maize bran (L. Saulnier, C. Marot, E. Chanliaud and J.-F. Thibault, Carbohydr. Polym. 26, 279-287 (1995)) and 0.8% of sugar beet pulp (V. Micard, G. M. G. C. Renard and J.-F. Thibault, Lebensm.-Wiss. u-Technol. 27, 59-66 (1994)). These materials are amongst the preferred sources of trans-ferulic acid. Since trans-ferulic acid is present esterified with cell-wall polysaccharides, hydrolysis is essential. Alkaline or acid hydrolysis is possible, but enzymic hydrolysis is preferred. Typically, the initial step is the partial enzymic hydrolysis of carbohydrates (arabinans, xylans, rhamnogalacturanans) to which trans-ferulate is linked, followed by the release of trans-ferulate from the oligosaccharide fragments by trans-**ferulic acid esterase** activity. In practice, both steps may occur simultaneously in the reaction mixture. Descriptions of representative laboratory-scale processes are available in the literature (for example see L. P. Christov and B. A. Prior, Enzyme Microb. Technol. 15, 460-475 (1993)); C. B. Faulds and G. Williamson, Appl. Microbiol. Biotechnol. 43, 1082-1087 (1995); C. B. Faulds, P. A. Kroon, L. Saulnier, J.-F. Thibault and G. Williamson, Carbohydrate Polymers 27, 187-190 (1995)). Phenolic acid-releasing enzymes have been reported from a number of microorganisms, including Streptomyces olivochromogenes (C. B. Faulds and G. Williamson, J. Gen. Microbiol. 137, 2337-2345 (1991)), Penicillium pinophilum (A. Castanares, S. I. McCrae and T. M. Wood, Enzyme Microb. Technol. 14, 875-884 (1992)), Neocallimastix spp. (W. S. Borneman, R. D. Hartley, W. H. Morrison, D. E. Akin and L. G. Ljungdahl, Appl. Microbiol. Biotechnol. 33, 345-35,1 (1990)), Schizophyllum commune (R. C. MacKenzie and D. Bilous, Appl. Envir. Microbiol. 54, 1170-1173 (1988)) and Aspergillus spp. (M. Tenkanen, J. Schuseil, J. Puls and K. Poutanen, J. Biotechnol. 18, 69-84 (1991); C. B. Faulds and G.

Williamson, Microbiology 140, 779-787 (1994)). A trans-**ferulic acid esterase** (XYLD) has been characterised from Pseudomonas fluorescens subsp. cellulosa, together with an arabinofuranosidase (XYLC) and an endoxylanase (XYLB; L. M. A. Ferreira, T. M. Wood, G. Williamson, C. B. Faulds, G. P. Hazlewood and H. J. Gilbert, Biochem. J. 294, 349-355 (1993)). The genes for all three enzymes have been isolated (G. P. Hazlewood and H. J. Gilbert, in "Xylans and Xylanases", eds. J. Visser, G. Beldman, M. A. Kusters-van Someren and A. G. J. Voragen, Elsevier, Amsterdam, pp 259-273 (1992)). All of these references are incorporated herein by reference.

Brief Summary Text - BSTX (51):

Thus, advantageously the trans-ferulic acid or a salt thereof may be provided by the action of trans-**ferulic acid esterase** on said ester. More particularly, it is advantageous to introduce a gene encoding said esterase into a host cell or organism which is being used in the methods of the invention. Thus, it is convenient to introduce a trans-**ferulic acid esterase** gene, such as the aforementioned XYLD gene, into a plant which is being used in the methods of the invention.

Other Reference Publication - OREF (29):

Faulds et al., "Release of Ferulic Acid from Wheat Bran by a **Ferulic Acid Esterase** (FAE-III) from Aspergillus niger," Appl. Microbiol. Biotechnol., 43:1082-1087 (1995).

Other Reference Publication - OREF (31):

Faulds et al., "The Purification and Characterization of 4-Hydroxy-3-Methoxycinnamic **(Ferulic) Acid Esterase** from Streptomyces olivochromogenes," J. Gen. Mcrobiol., 137:2339-2345 (1991).

Other Reference Publication - OREF (32):

Castanares et al., "Purification and Properties of a Feruloyl/.rho.-**Coumaroyl Esterase** from the Fungus Penicillium phiophilum," Enzyme Microb. Technol., 14:875-884 (1992).

Other Reference Publication - OREF (33):

Borneman et al., "Feruloyl and .rho.-**Coumaroyl Esterase** from Anaerobic Fungi in Relation to Plant Cell Wall Degradation," Appl. Microbiol. Biotechnol., 33:345-351 (1990).

Other Reference Publication - OREF (34):

MacKenzie et al., "**Ferulic Acid Esterase** Activity from Schizophyllum commune," Appl. Enviorn. Microbiol., 54:1170-1173 (1988).

Other Reference Publication - OREF (35):

Tenkanen et al., "Production, Purification and Characterization of an

Esterase Liberating Phenolic Acids from Lignocellulosics," J. Biotechnol., 18:69-84 (1991).

Other Reference Publication - OREF (36):

Faulds et al., "Purification and Characterization of a **Ferulic Acid Esterase** (FAE-III) from Aspergillus niger: Specificity for The Phenolic Moiety and Binding to Microcrystalline Cellulose," Microbiol., 140:779-787 (1994).

US-PAT-NO: 6638554

DOCUMENT-IDENTIFIER: US 6638554 B1

TITLE: Continuous production of an instant corn flour for arepa and tortilla, using an enzymatic precooking

DATE-ISSUED: October 28, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Rubio; Manuel J.	Miami Beach	FL	N/A	N/A
Contreras; Roberto	Guadalupe	N/A	N/A	MX
Rubio; Felipe	Edinburg	TX	N/A	N/A

APPL-NO: 10/ 231291

DATE FILED: August 30, 2002

US-CL-CURRENT: 426/508, 426/463 , 426/464 , 426/622 , 426/626

ABSTRACT:

Precooked and partially-debranned corn flour is continuously produced by an enzymatic precooking using a commercial xylanase as a processing aid. The low-temperature and near neutral-pH precooking with a xylanase effected a partial bran hydrolysis while avoiding excessive pregelatinization, reduced washing, and corn solid loss in wastewater. Moisture content is then stabilized, followed by milling and drying at a high-temperature and short-time to produce a controlled gelatinization in the ground kernel, cooling and further drying the dried-ground particle. A fine particle size or flour is separated and recovered from the coarser particle which is also segregated to partially isolate a bran fraction for animal feed or integral flour, remilling and sieving the coarser particle to produce an instant corn flour for arepa, and admixing the fine particle with lime to obtain a masa flour for tortilla and other snack foods.

15 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

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Brief Summary Text - BSTX (11):

In addition, the Enzyme Technical Association (ETA, 1999; FDA, 1998) classified as carbohydrases the following hemicellulases (trivial name): a)

endoenzymes (EC 3.2.1.32=1,3-.beta.-xylanohydrolase, 78=mannanohydrolase and 99=arabinohydrolase) and b) exoenzymes only attack branches on the xylose-polymer (pentosan), producing xylo-oligomers (EC 3.2.1.55=.alpha.-L-arabinofuranosidase, glucuronic-acid glycosilase and **ferulic-acid esterase**).

US-PAT-NO: 6620985

DOCUMENT-IDENTIFIER: US 6620985 B1

TITLE: PAD4 nucleic acid compositions from Arabidopsis and methods therefor

DATE-ISSUED: September 16, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Glazebrook; Jane	San Diego	CA	N/A	N/A
Jirage; Dayadevi	Hyattsville	MD	N/A	N/A
Tootle; Tina L.	Boston	MA	N/A	N/A
Zhou; Nan	Durham	NC	N/A	N/A
Feys; Bart Julienne Frans	Norwich	N/A	N/A	GB

APPL-NO: 09/ 434840

DATE FILED: November 4, 1999

PARENT-CASE:

The present application claims priority to Provisional Application No. 60/183,020, filed Nov. 12, 1998.

US-CL-CURRENT: 800/278, 435/320.1 , 435/419 , 536/23.6

ABSTRACT:

PAD4 compositions from Arabidopsis, including genomic and cDNA nucleic acid sequences, and methods for using said compositions in plants are provided. PAD4 from Arabidopsis is demonstrated to be regulatory and is required upstream from salicylic acid in the signal transduction pathway leading from infection to activation of defense responses.

10 Claims, 36 Drawing figures

Exemplary Claim Number: 1,5

Number of Drawing Sheets: 36

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Drawing Description Text - DRTX (29):

FIGS. 11A-11B. Alignment of Certain Segments of the PAD4 Amino Acid Sequence with Those of Various Lipases and a Hydrolase. Amino acid sequences listed above the numbered line that heads each group of six aligned sequences

represent majority sequences. The first through sixth amino acid sequence, respectively, in each group of six aligned sequences are identified by the amino acid positions leftmost in each line as follows: the first amino acid sequences--having positions 147, 186, 217, and 250 leftmost--are from a triacyl glycerol lipase from Fusarium heterosporum (GenBank Accession number S77816; SEQ ID NO.:29), the second amino acid sequences--having positions 209, 248, 284, and 317 leftmost--are from a triacyl glycerol lipase from Rhizomucor miehei (GenBank accession number A34959; SEQ ID NO.:30), the third amino acid sequences--having positions 239, 278, 314, and 347 leftmost--are from a lipase from Rhizopus niveus (GenBank accession number AB013496; SEQ ID NO.:31), the fourth amino acid sequences--having positions 139, 178, 209, and 243 leftmost--are from a lipase from Thermomyces lanuginosus (GenBank accession number AF054513; SEQ ID NO.:32), the fifth amino acid sequences--having positions 75, 113, 144, and 183 leftmost--are from PAD4 (SEQ ID NO.:2), and the sixth amino acid sequences--having positions 125, 164, 195, and 235 leftmost--are from a **ferulic acid esterase** A from Aspergillus niger (Gen Bank accession number Y09330; SEQ ID NO.: 33).

Drawing Description Text - DRTX (45):

FIGS. 19A-19C. Alignment of Amino Acid Segment of PAD4 Surrounding a Serine (FIG. 19A), an Aspartic Acid (FIG. 19B), and a Histidine (FIG. 19C) of Putative Lipase Catalytic Triad with those of Various Lipases, an Esterase, and EDS1 from Arabidopsis. Residues of the putative lipase catalytic triad consisting of a serine, a histidine, and an aspartate are indicated by arrows. RhizoTGL=triacylglycerol lipase (EC 3.1.1.3) precursor 1 from Rhizomucor miehei; FusaTGL=triacylglycerol lipase (EC 3.1.1.3) from Fusarium heterosporum; Rhizolip=triacylglycerol lipase (EC 3.1.1.3) precursor 1 from Rhizomucor niveus; Thermolip=lipase from Thermomyces lanuginosus; AspFAE=**ferulic acid esterase** A from Aspergillus niger; AtEDS1=Arabidopsis thaliana EDS 1; AtPAD4=Arabidopsis thaliana PAD4. Invariant residues are indicated in bold letters and conserved amino acids are underlined. The numbering of AtPAD4 residues follows the numbering of SEQ ID NO.:55.

Detailed Description Text - DETX (113):

However, PAD4 is also similar to **ferulic acid esterase** from Aspergillus niger (FIG. 11A, FIG. 11B, FIG. 19A, FIG. 19B, and FIG. 19C). Consequently, it is also possible that PAD4 acts on a substrate that is non-lipid in nature, such as ferulic acid.

US-PAT-NO: 6616953

DOCUMENT-IDENTIFIER: US 6616953 B2

TITLE: Concentrated spent fermentation beer or saccharopolyspora erythraea activated by an enzyme mixture as a nutritional feed supplement

DATE-ISSUED: September 9, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Fidler; Daniel J.	Gurnee	IL	N/A	N/A
Lampel; Jay Sanford	Mundelein	IL	N/A	N/A
Weyant; Daniel B.	Winthrop Habor	IL	N/A	N/A

APPL-NO: 10/ 036908

DATE FILED: December 21, 2001

PARENT-CASE:

This application claims priority to the provisional application Serial No. 60/259,163 filed on Jan. 2, 2001.

US-CL-CURRENT: 426/2, 426/31 , 426/52 , 426/53 , 426/61 , 426/807

ABSTRACT:

A feed additive composition which contains enzyme-treated, concentrated spent fermentation beer of Saccharopolyspora erythraea; a method for making a feed additive containing enzyme-treated concentrated spent fermentation beer of Saccharopolyspora erythraea; a process for improving the nutritive value of spent fermentation beer of Saccharopolyspora erythraea by enzymatic treatment; a method for feeding livestock with a feed containing an enzyme-treated, concentrated spent fermentation beer of Saccharopolyspora erythraea additive and a process for improving poultry feed conversion, breast meat yield and intestinal strength by feeding a feed containing an enzyme-treated, concentrated spent fermentation beer of Saccharopolyspora erythraea additive are disclosed. Preferably, the spent fermentation beer of Saccharopolyspora erythraea is treated with a cellulase and at least one glycosidase, and then concentrated.

44 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (10):

Some enzymes have been clearly recognized in the marketplace for their value as additives in animal feeds: xylanse, .beta.-glucanase, enzymes that cleave phosphorus from phytic acid, hemicellulates (as disclosed in U.S. Pat. No. 6,162,473), **ferulic acid esterase** (as disclosed in U.S. Pat. No.6,143,543) and mannanase. In addition to enzyme feed additives, small molecules such as aminocarboxylic acid derivatives as disclosed in U.S. Pat. No. 6,166,086 are also useful, and marine mammals treated with proteolytic enzymes have also been disclosed (U.S. Pat. No. 6,153,251). Fermentation products are also known as feed additives, such as a fermented formula feed obtainable from mixing a soybean feed material with wheat as disclosed in U.S. Pat. No. 6,090,416; and liquid Saccharopolyspora solubles. However, there is still a need for inexpensive and more efficient additives.

US-PAT-NO: 6607902

DOCUMENT-IDENTIFIER: US 6607902 B2

TITLE: Cell-wall degrading enzyme variants

DATE-ISSUED: August 19, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Schr.o slashed.der Glad;	Ballerup	N/A	N/A	DK
Sanne O	V.ae butted.rl.o	N/A	N/A	DK
Andersen; Carsten	slashed.se	N/A	N/A	DK
Schulein; Martin	late of Copenhagen	N/A	N/A	DK
Frandsen; Torben Peter	Frederiksberg			

APPL-NO: 09/ 910505

DATE FILED: July 19, 2001

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims, under 35 U.S.C. 119, priority of Danish application nos. PA 2000 01117, filed Jul. 19, 2000, PA 2001 00705, filed May 4, 2001, and PA 2001 00734, filed May 10, 2001, and the benefit of U.S. provisional application No. 60/290,724, filed May 14, 2001, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	2001 00705	May 4, 2000
DK	2000 01117	July 19, 2000
DK	2001 00734	May 10, 2001

US-CL-CURRENT: 435/232, 435/263 , 435/264 , 435/267 , 510/300 , 536/23.1 , 536/23.2

ABSTRACT:

A variant of a cell-wall degrading enzyme having a beta-helix structure, which variant holds at least one substituent in a position determined by identifying all residues potentially belonging to a stack; characterising the stack as interior or exterior; characterising the stack as polar, hydrophobic or aromatic/heteroaromatic based on the dominating characteristics of the parent or wild-type enzyme stack residues and/or its orientation relative to the beta-helix (interior or exterior); optimizing all stack positions of a stack either to hydrophobic aliphatic amino acids, hydrophobic aromatic or polar amino acids by allowing mutations within one or all positions to amino

acids belonging to one of these groups; measuring thermostability of the variants by DSC or an application-related assay such as a Pad-Steam application test; and selecting the stabilized variants. Variant of a wild-type parent pectate lyase (EC 4.2.2.2) having the conserved amino acid residues D111, D141 or E141, D145, K165, R194 and R199 when aligned with the pectate lyase comprising the amino acid sequence of SEQ ID NO: 2 are preferred.

31 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

---------- KWIC ---------

Brief Summary Text - BSTX (26):

Endo- and exo-xylanases and accessory enzymes such as glucuronidases, arabinofuranosidases, acetyl xylan **esterase and ferulic** acid or **coumaric acid esterase** have been summarized by Kormelink (1992, Ph.D.-thesis, University of Wageningen, The Netherlands). They are produced by a wide variety of micro-organisms and have varying temperature and pH optima.

US-PAT-NO: 6602842

DOCUMENT-IDENTIFIER: US 6602842 B2

TITLE: Cleaning compositions containing plant cell wall degrading enzymes and their use in cleaning methods

DATE-ISSUED: August 5, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cuperus; Roelck A.	Amsterdam	N/A	N/A	NL
Herweijer; Margareta A.	Den Haag	N/A	N/A	NL
Van Ooijen; Albert J. J.	Voorburg	N/A	N/A	NL
Van Schouwen; Dick J.	Vlaardingen	N/A	N/A	NL

APPL-NO: 09/ 828374

DATE FILED: April 5, 2001

PARENT-CASE:

The present application is a Divisional of U.S. patent application Ser. No. 08/737,970, filed Nov. 27, 1997, now U.S. Pat. No. 5,872,091, which is a 371 of PCT/EP95/02380, filed Jun. 19, 1995, and EP 94201741.9, filed Jun. 17, 1994.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
EP	94201741	June 17, 1994

US-CL-CURRENT: 510/392, 510/276 , 510/320

ABSTRACT:

Novel cleaning compositions comprising cell wall degrading enzymes are disclosed having pectinases and/or hemicellulases and optionally cellulases. The compositions are particularly suitable for removing stains of vegetable origin, especially from textiles. Although compositions having only one type of such enzymes axe part of the invention (excluding cellulases alone), preferred embodiments have a mixture of cell wall degrading enzyme activities to allow for a concerted action against the fibrous mass which usually constitutes a stain of vegetable origin.

29 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (25):

Endo- and exo-xylanases and accessory enzymes such as glucuronidases, arabinofuranosidases, acetyl xylan **esterase and ferulic** acid or **coumaric acid esterase** have been summarized by Kormelink (1992, Ph.D.-thesis, University of Wageningen, The Netherlands). They are produced by a wide variety of micro-organisms and have varying temperature and pH optima.

Claims Text - CLTX (5):

5. The laundry detergent composition of claim 1, further comprising a second hemicellulase, wherein said second hemicellulase is selected from the group consisting xylanases, arabinofuranosidases, acetyl xylan **esterases, glucuronidases, ferulic acid esterases, coumaric acid esterases,** endo-galactanases, mannanases, lichenases, endo-arabinases, exo-arabinanases, and exo-galactanases.

Claims Text - CLTX (18):

18. The method of claim 15, wherein said laundry detergent composition comprises at least one pectinase selected from the group consisting of pectin esterases, pectin lyases, exopolygalacturonases, endopolygalacturonases, and rhamnogalacturonases, and a hemicellulase, wherein said hemicellulase is a microbial mannanse is obtained from strain C11SB.G17 (CBS 480.95), and a second hemicellulase selected from the group consisting of consisting xylanases, arabinofuranosidases, acetyl xylan **esterases, glucuronidases, ferulic acid esterases, coumaric acid esterases,** endo-galactanases, mannanases, lichenases, endo-arabinases, exo-arabinanases, and exo-galactanases.

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TITLE: **Phenolic acid esterases,** coding sequences and methods

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APPL-NO: 09/ 603311

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PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a Continuation-in-part of U.S. patent application No. 09/390,234, filed Sep. 3, 1999, now U.S. Pat. No. 6,365,390 which application claims priority from U.S. Provisional Application No. 60/099,136, filed Sep. 4, 1998.

US-CL-CURRENT: 435/267, 435/136 , 435/19 , 435/196 , 435/320.1 , 530/350 , 536/23.2

ABSTRACT:

Described herein are four **phenolic acid esterases,** three of which correspond to domains of previously unknown function within bacterial xylanases, from XynY and XynZ of Clostridium thermocellum and from a **feruloyl esterase** of Ruminococcus. The fourth specifically exemplified **phenolic acid esterase** is a protein encoded within the genome of Orpinomyces PC-2. The amino acids of these polypeptides and nucleotide sequences encoding them are provided. Recombinant host cells, expression vectors and methods for the recombinant production of **phenolic acid esterases** are also provided. Further provided are methods for improving nutrient availability and ferulic acid availability when food or feed, or other material is treated with a **phenolic acid esterase,** desirably in combination with a xylanase.

36 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

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Abstract Text - ABTX (1):

Described herein are four **phenolic acid esterases,** three of which correspond to domains of previously unknown function within bacterial xylanases, from XynY and XynZ of Clostridium thermocellum and from a **feruloyl esterase** of Ruminococcus. The fourth specifically exemplified **phenolic acid esterase** is a protein encoded within the genome of Orpinomyces PC-2. The amino acids of these polypeptides and nucleotide sequences encoding them are provided. Recombinant host cells, expression vectors and methods for the recombinant production of **phenolic acid esterases** are also provided. Further provided are methods for improving nutrient availability and ferulic acid availability when food or feed, or other material is treated with a **phenolic acid esterase,** desirably in combination with a xylanase.

TITLE - TI (1):

Phenolic acid esterases, coding sequences and methods

Brief Summary Text - BSTX (4):

The field of the present invention is the area of enzymes which degrade plant cell walls, and certain other substrates, in particular, the **phenolic acid esterases, feruloyl esterases** and/or **coumaroyl esterase,** and methods for using them in food compositions, feed compositions and supplements, nutriceuticals and in pulping.

Brief Summary Text - BSTX (5):

Plant cell wall material is one of the largest sources of renewable energy on earth. Plant cell walls are composed mainly of cellulose, hemicelluloses, lignin and pectin. Arabinoxylan is one of the main constituents of hemicelluloses. It is composed of a chain of .beta.(1.fwdarw.4) linked xylose units that are substituted by arabinose, acetate, and glucuronic acid. The arabinose has ester linked ferulic and p-coumaric acids [Bomeman et al. (1993) In: Hemicellulose and Hemicellulases, Coughlan and Hazlewood, Eds., pp. 85-102]. Ferulic acid has been shown to link hemicellulose and lignin [Ralph et al. (1995) Carbohydrate Research 275:167-178]. **Feruloyl esterases** are involved in breaking the bond between the arabinose and ferulic acid, thus releasing the covalently bound lignin from hemicelluloses. **Feruloyl esterases** have been found in many bacteria as well as fungi, but have not been extensively studied nor is there much sequence data available [Christov and Prior (1993) Enzyme. Microb. Technol. 15(6):460-75].

Brief Summary Text - BSTX (6):

Clostridium thermocellum is a gram-positive bacterium that produces a multienzymatic structure termed the cellulosome. The cellulosome is one of the most active cellulose degrading complexes described to date. The cellulosome has a multi-polypeptide structure, including a scaffolding subunit which has nine cohesins binding to nine catalytic subunits, a dockerin domain for attachment to the cell wall, and a cellulose binding domain [Felix and Ljungdahl (1993) Annu. Rev. Microbiol. 47:791-819]. The catalytic subunits include endoglucanase, cellobiohydrolase, lichenase, and xylanase, many of which have been cloned and sequenced. They all have multidomain structures that include at least a dockerin domain for binding to the scaffolding domain, a linker, and a catalytic domain. They may also contain cellulose binding domains and fibronectin-like domains. There are reports that some enzymatic components may have more than one catalytic domain. Two of these are xylanase Y [XynY, Fontes et al. (1995) Biochem. J. 307:151-158] and xylanase Z [XynZ, Grepinet et al. (1988) J. Bacteriol. 170(10):4582-8]. XynY has a C-terminal domain whereas XynZ N-terminal domain without any functions determined. Although enzymes with dual catalytic domains (xylanase and .beta.glucanase) have been found in other bacteria [Flint et al. (1993) J. Bacteriol. 175:2943-2951] neither **phenolic acid esterase** nor bifunctional enzymes have been found in C. thermocellum.

Brief Summary Text - BSTX (7):

There is a need in the art for **phenolic acid esterases, feruloyl esterases** and/or **coumaroyl esterases** in pure form which degrade plant cell wall materials, and certain other substrates, for DNA encoding these enzymes to enable methods of producing ferulic acid and/or coumaric acid, as well as facilitating degradation of plant cell wall materials in the context of human, animal, fish or shellfish food, the pulping industry and in the area of nutriceuticals.

Brief Summary Text - BSTX (9):

The present invention provides methods for improving nutrient availability in foods, especially plant-derived feedstuffs and foodstuffs with a significant non-starch polysaccharide content and/or with poorly digestible fiber. The methods comprise the step of combining the foodstuff or feedstuff with a **feruloyl esterase** as provided herein, desirably together with a xylanase, for example, the xylanase (XynA) protein derived from Orpinomyces PC-2. These enzymes can be prepared from their natural sources or the recombinant enzymes can be prepared using the teachings provided herein and in United States Patent No. 5,824,533 (Li et al., 1998) for the xylanase A of Orpinomyces PC-2. A foodstuff or feedstuff is combined with **feruloyl esterase** at a ratio of 0.1 to 200 units per kg dry weight of foodstuff or feedstuff. Where the xylanase A protein is also added, the xylanase ratio is 100 to 25,000 units per kg. An advantageous range is 500 to 10,000.U/kg or 1000 to 5000 U/kg. Where a beverage or liquid food or feed formulation is treated with **feruloyl esterase or feruloyl esterase** and xylanase A, the ratios are the same, with the calculation based on the dry weight of solids in the beverage or other liquid composition for consumption by a human or an animal.

Brief Summary Text - BSTX (10):

The present invention further provides methods for improved pulping of plant material or recycled fiber materials, wherein the improvement comprises the step of adding a **feruloyl acid esterase** to the pulping mixture and incubating under conditions allowing enzymatic action of the esterase on the non-starch polysaccharides in the mixture. The mixtures desirably contain a combination of **feruloyl esterase** and xylanase A. In pulping, the esterase is present in the pulping mixture at a ratio of 0.1 to 200 U/kg dry weight of pulp or fiber, and where xylanase A is present, the xylanase is present at a ratio of from 100 to 25,000 U/kg, desirably from 500 to 10,000, or from 1000 to about 5000 U/kg fiber or pulp dry weight.

Brief Summary Text - BSTX (11):

The present invention further provides compositions comprising a **feruloyl esterase** protein. Desirably the composition further comprises a xylanase optionally xylanase A from Orpinomyces PC-2. These compositions can be liquid (non-concentrated solution or suspension) or dry (e.g., freeze-dried). The user then adds the liquid composition to food, feed or fiber, or the dry composition can be reconstituted before or upon addition to a food, feed or pulp.

Brief Summary Text - BSTX (12):

The present invention further provides methods for improving the availability of ferulic acid from plant material for human, animal, fish or shellfish nutrition or health benefit. A **feruloyl esterase** composition described herein or a combination of a **feruloyl esterase** together with xylanase A is (are) added to a plant-derived foodstuff or feedstuff prior to consumption. Alternatively, a composition containing a **feruloyl esterase digest or a feruloyl esterase**--xylanase A digest of plant cell wall-containing material can be provided for use as a nutritional or nutriceutical supplement.

Brief Summary Text - BSTX (13):

Herein are described novel **phenolic acid esterases, having feruloyl esterase and coumaroyl esterase** activities, and coding sequences for same.

Brief Summary Text - BSTX (14):

One **phenolic acid esterase** corresponds to a domain of previously unknown function from xylanase Y of Clostridium thermocellum. The recombinantly expressed domain polypeptide is active and has an amino acid sequence as given in FIG. 1 as "XynY_Clotm." The nucleotide sequence encoding the esterase polypeptide is given in Table 5, nucleotides 2383-3219, exclusive of translation start and stop signals. See also SEQ ID NOs:11 and 12.

Brief Summary Text - BSTX (15):

A second **phenolic acid esterase** corresponds to a domain of previously unknown function of xylanase Z from C. thermocellum. The amino acid sequence of the esterase domain, which also is active when expressed as a recombinant polypeptide, is given in FIG. 1 as "XynZ_Clotm." The nucleotide sequence

encoding this polypeptide is given in Table 6, nucleotides 58-858. Further described is a **phenolic acid esterase** polypeptide additionally comprising a cellulose binding domain. A specifically identified cellulose binding domain has an amino acid sequence as given in Table 6, 289-400, with a corresponding coding sequence as given in Table 6, nucleotides 867-1200. See also SEQ ID NOs:13 and 14.

Brief Summary Text - BSTX (16):

An additional object of the present invention is a **phenolic acid esterase (i.e., a feruloyl esterase)** derived from a previously uncharacterized portion of a Ruminococcus xylanase (See FIG. 1). The coding (nucleotides 2164-2895, exclusive of translation start and stop signals) and deduced amino acid sequences (amino acids 546-789) are given in Table 10. See also SEQ ID NOs:15 and 16.

Brief Summary Text - BSTX (17):

A feruloyl **(phenolic acid) esterase** is prepared from the anaerobic fungus Orpinomyces PC-2. The coding sequence and deduced amino acid sequences of the mature esterase protein are given in Table 9, and the purification of the Orpinomyces enzyme is described herein below. See also SEQ ID NOs:17 and 18.

Brief Summary Text - BSTX (18):

Recombinant production of the phenolic (especially **ferulic) acid esterases** is described. Escherichia coli, Bacillus subtilis, Streptomyces sp., Saccharomyces cerevisiae, Aureobasidium pullulans, Pichia pastoris, Trichoderma, Aspergillus nidulans or any other host cell, including plants, suitable for the production of a heterologous protein can be transfected or transformed with an expression vector appropriate for the chosen host. Compatible combinations of vectors and host cells are well known in the art, as are appropriate promoters to be used to direct the expression of a particular coding sequence of interest. The recombinant host cells are cultured under conditions suitable for growth and expression of the **phenolic acid esterase,** and the recombinant esterase is then collected or the recombinant host cells in which the esterase has been produced are collected. The coding sequence of the esterase can be operably linked to a nucleotide sequence encoding a signal peptide which is known in the art and functional in the desired host cell if secretion of the esterase into the culture medium is desired. In that case, the culture medium serves as the source of esterase after growth of the host cells.

Brief Summary Text - BSTX (19):

It is recognized by those skilled in the art that the DNA sequences may vary due to the degeneracy of the genetic code and codon usage. All DNA sequences which encode a **phenolic acid esterase** polypeptide having a specifically exemplified amino acid sequence are included in this invention, including DNA sequences encoding them having an ATG preceding the coding region for the mature protein and a translation termination codon (TAA, TGA or TAG) after the coding sequence.

Brief Summary Text - BSTX (20):

Additionally, it will be recognized by those skilled in the art that allelic variations may occur in the **phenolic acid esterase** polypeptide coding sequences which will not significantly change activity of the amino acid sequences of the polypeptides which the DNA sequences encode. All such equivalent DNA sequences are included within the scope of this invention and the definition of a **phenolic acid esterase**. The skilled artisan will understand that the amino acid sequence of an exemplified **phenolic acid esterase** polypeptide and signal peptide(s) can be used to identify and isolate additional, nonexemplified nucleotide sequences which will encode functional equivalents to the polypeptides defined by the amino acid sequences given herein or an amino acid sequence of greater than 40% identity thereto and having equivalent biological activity. All integer percents between 40 and 100 are encompassed by the present invention. DNA sequences having at least about 75% homology to any of the **ferulic acid esterases** coding sequences presented herein and encoding polypeptides with the same function are considered equivalent to thereto and are included in the definition of "DNA encoding a **phenolic acid esterase.**" Following the teachings herein, the skilled worker will be able to make a large number of operative embodiments having equivalent DNA sequences to those listed herein.

Brief Summary Text - BSTX (21):

Feruloyl esterase proteins are characterized by at least a portion having from at least about 40% amino acid sequence identity with an amino acid sequence as given in SEQ ID NO:18, amino acids 227 to 440 (within the **feruloyl esterase** protein of Orpinomyces PC-2. All integer percent identities between 40 and 100% are also contemplated. Similarly, **feruloyl esterase** proteins can have from about 40% to about 100% identity with an amino acid sequence from the group comprising amino acids 581 to 789 of SEQ ID NO:16, amino acids 845 to 1075 of SEQ ID NO:12, amino acids 69 to 286 of SEQ ID NO:14, amino acids 69 to 307 of SEQ ID NO:14, and amino acids 69 to 421 of SEQ ID NO:14. Specifically exemplified **feruloyl esterases** are characterized by amino acid sequences from the group comprising amino acids 227 to 440 of SEQ ID NO:18, amino acids 581 to 789 of SEQ ID NO:16, amino acids 845 to 1075 of SEQ ID NO:12, amino acids 69 to 286 of SEQ ID NO:14, amino acids 69 to 307 of SEQ ID NO:14, and amino acids 69 to 421 of SEQ ID NO:14. **Feruloyl esterase** proteins include those having the following amino acid sequences: SEQ ID NO:18, amino acids 1 to 530; SEQ ID NO:12, amino acids 795 to 1077; SEQ ID NO:16, amino acids 546 to 789; SEQ ID NO:14, amino acids 20 to 286; SEQ ID NO:14, amino acids 20 to 307; and SEQ ID NO:14, amino acids 20 to 421.

Brief Summary Text - BSTX (22):

Specifically exemplified nucleotide sequences encoding the **feruloyl esterase** proteins include the following: SEQ ID NO:17, nucleotides 1 to 1590; SEQ ID NO:11, nucleotides 2582-3430; SEQ ID NO:15, nucleotides 2164 to 2895; SEQ ID NO:13, nucleotides 158 to 958; SEQ ID NO:13, nucleotides 158 to 1021; SEQ ID NO:13, nucleotides 158 to 1363.

Brief Summary Text - BSTX (23):

The **phenolic acid esterase** coding sequences, including or excluding that encoding a signal peptide, can be used to express a **phenolic acid esterase** in recombinant fungal host cells or plant cells as well as in bacteria, including without limitation, Bacillus spp., Streptomyces sp. and Escherichia coli. Any host cell in which the signal sequence is expressed and processed may be used. Preferred host cells are Aureobasidium species, Aspergillus species, Trichoderma species and Saccharomyces cerevisiae, as well as other yeasts known to the art for fermentation, including Pichia pastoris [See, e.g., Sreekrishna, K. (1993) In: Industrial Microorganisms: Basic and Applied Molecular Genetics, Baltz, R. H., et al. (Eds.) ASM Press, Washington, D.C. 119-126]. Filamentous fungi such as Aspergillus, Trichoderma, Penicillium, etc. are also useful host organisms for expression of the DNA of this invention. [Van den Handel, C. et al. (1991) In: Bennett, J. W. and Lasure, L. L. (Eds.), More Gene Manipulations in Fungi, Academy Press, Inc., New York, 397-428].

Drawing Description Text - DRTX (2):

FIG. 1 shows amino acid sequence alignment of the exemplified **phenolic acid esterases**. Sequences are xylanase Z [XynZ_Clotm, Grepinet et al. (1988) supra], xylanase Y [XynY_Clotm, Fontes et al. (1995) supra] of C. thermocellum, xylanase A (XynA_Rumin) of a Ruminococcus sp, and a hypothetical 44-kDa protein of E coli (Genbank Accession Number P31471) (SEQ ID NO:19). Amino acid numbering was the same as in the databases. Dots represent gaps introduced to optimize alignment, and are treated as mismatched in calculations of sequence relatedness (similarity or identity). The partial amino acids are derived from SEQ ID NO:20, SEQ ID NO:12, SEQ ID NO:16, SEQ ID NO:19 and SEQ ID NO:18.

Drawing Description Text - DRTX (4):

FIG. 3 illustrates the results of Superose 6 gel filtration of proteins eluted from Avicel adsorption of C. thermocellum culture supernatant. Fractions (0.5 ml) were collected and assayed for protein and **feruloyl esterase** activity. Molecular mass standards (Sigma Chemical Company, St. Louis, Mo.) including blue dextran (2,000 kDa), catalase (232 kDa), ovalbumin (43 kDa), and ribonuclease A (13.7 kDa) were run under identical conditions and their elution positions were indicated.

Drawing Description Text - DRTX (7):

FIGS. 6A and 6B, respectively, illustrate the effects of temperature and pH on **feruloyl esterase** activity of the C. thermocellum XynZ FAE/CBD. Buffer used for evaluating temperature effects was 50 mM sodium citrate, pH 6.0. Assays mixtures with a pH range from 2 to 10 were formulated by using a universal phosphate buffer system.

Drawing Description Text - DRTX (8):

FIG. 7 illustrates the results of SDS-PAGE analysis of the purified **feruloyl esterase** from the culture supernatant of Orpinomyces sp. strain PC-2 (lane 1); molecular mass markers are in lane 2.

Drawing Description Text - DRTX (9):
FIGS. 8A and 8B show the temperature and pH activity profiles, respectively, of the Orpinomyces sp. strain PC-2 **feruloyl esterase**.

Drawing Description Text - DRTX (10):
FIG. 9 shows alignment of protein sequences exhibiting homology to the Orpinomyces **feruloyl esterase**. Sequences are: faea_orpin, Orpinomyces sp. strain PC-2 FaeA; xyna_rumin, xylanase from Ruminococcus sp. (Genbank Accession Number S58235); yiel_ecoli hypothetical 44kDa protein from E. coli (Genbank Accession Number P31471); xyny_clotm, xylanase Y from C. thermocellum (Genbank Accession Number P51584); xynz_clotm, xylanase Z from C. thermocellum (Genbank Accession Number M22624); dppv_asprf, dipeptidyl peptidase from A. fumigatus (Genbank Accession Number L48074) (SEQ ID NO:20). The partial sequences are taken from. SEQ ID NO:18, SEQ ID NO:16, SEQ ID NO:22, SEQ ID NO:12, SEQ ID NO:14 and SEQ ID NO:20.

Detailed Description Text - DETX (6):
Genes encoding **feruloyl esterase** (faeA) have been cloned from Aspergillus niger and Aspergillus tubingensis and the deduced amino acid sequences bear close similarity to lipases [de Vries et al. (1997) Appl. Environ. Microbiol. 63:4638-4644]. Expression of these gene products is regulated by the xlnR gene product [van Peij et al. (1998) Appl. Environ. Microbiol. 64:3615-3619]. Other genes include the xylD gene from Pseudomonas fluorescens subsp. cellulosa, the gene product of which has a higher specificity for acetyl groups than feruloyl groups [Ferreira et al. (1993) Biochemical J. 294:349-355] and two genes from Butyrivibro fibrisolvens termed cinA and cinB [Dalrymple and Swadling (1997) Microbiology 143:1203-1210; Dalrymple et al. (1996) FEMS Microbiol. Lett. 143:115-120]. These genes are believed to be regulated by the cinR gene product which may itself be regulated by FAXX [Dalrymple and Swadling (1997) supra]. Esterase activity has also been studied in Streptomyces olivochromogenes [Faulds and Williamson (1991) J. Gen. Microbiol. 137:2339-2345], Schizophyllum commune [MacKenzie and Bilous (1988) Appl. Environ. Microbiol. 54:1170-1173], Penicillium pinophillum [Castanares and Wood (1992) Biochem. Soc. Trans. 20:275S], and Fibrobacter succinogenes [McDermid et al. (1990) Appl. Environ. Microbiol. 56:127-132].

Detailed Description Text - DETX (7):
As described herein, **feruloyl esterases** are found as part of xylanases from the Clostridium thermocellum cellulosome or as an individual enzyme, for example, from Orpinomyces sp. PC-2. Xylanases Y and Z from C. thermocellum are composed of a xylanase domain, a linker domain, and other domains as well as a domain to which no function has been assigned. We found partial sequence homology between these enzyme and the **feruloyl esterase** of Orpinomyces in the region of the previously unknown domains and demonstrated that these domains indeed encode **feruloyl esterases**. Herein, we also report the purification, cloning, and partial characterization of the **feruloyl esterase** from Orpinomyces sp. strain PC-2.

Detailed Description Text - DETX (8):

Anaerobic fungi produce high levels of **phenolic esterases** [Borneman and Akin (1990) In: Microbial and Plant Opportunities to Improve Lignocellulose Utilization by Ruminants. D. E. Akin, L. G. Ljungdahl, J. R. Wilson, and P. J. Harris (Eds.). Elsevier Science Publishing Co. New York, pp. 325-340] and two **feruloyl esterases** of the anaerobic fungus Neocallimastix MC-2 were purified and characterized [Borneman et al. (1992) Appl. Environ. Microbiol. 58:3762-3766]. A cDNA coding for a **feruloyl esterase** (FaeA) of the anaerobic fungus Orpinomyces PC-2 was cloned and sequenced by the present inventors. FASTA and BLAST searches showed that the catalytic domain of the Orpinomyces FaeA was over 30% identical to sequences coding for unknown domains (UD) in the databases including the carboxy terminal region of XynY Fontes et al. (1995) supra], the amino terminal region of XynZ [Grepinet et al. (1988) supra], a hypothetical polypeptide of E. coli (Genbank Accession Number P31471), and the carboxy terminal region of a Ruminococcus xylanase [Genbank Accession No. S58235] (FIG. 1). No function had been previously assigned to the sequences homologous to the Orpinomyces FaeA. XynY consists of multiple domains including a family F xylanase domain, followed by a putative thermostability domain, a dockerin, and the UD [Fontes et al. (1995) supra]. Similarly, XynZ is also multi-domain enzyme containing the UD, a family VI cellulose binding domain, a dockerin, and a family 10 xylanase domain [Grepinet et al. (1988) supra; Tomme et al. (1995) In: Enzymatic Degradation of insoluble Carbohydrates. J. N. Saddler, M. H. Panner (Eds.), ACS Symposium Series, American Chemical Society, Washington, D.C., pp. 142-163]. Both XynY and XynZ are believed to be components of the cellulosome (FIG. 2). The Orpinomyces FaeA together with those homologous sequences, however, failed to show significant homology to the recently published **feruloyl esterases** (FaeA) of Aspergillus niger and A. tubingensis [de Vries et 5 al. (1997) supra]. The sequence analysis implies that a new type of **feruloyl esterase** is encoded by the Orpinomyces cDNA and the homologous sequences described above.

Detailed Description Text - DETX (9):

We have determined that C. thermocellum produces **feruloyl esterase** activity under the conditions when the cellulosome production is induced. The bacterium was cultivated on low concentration (0.2%, w/v) of Avicel, and under this growth condition, most of the substrate was consumed and cellulosomes released into culture medium, as indicated by the activities on Avicel and xylan (Table 2). Most of the **feruloyl esterase** activity (97.9%) was found in the culture medium (Table 2). It is well documented that cellulosomes of C. thermocellum are readily adsorbed to cellulose [Morag et al. (1992) Enzyme Microb. Technol. 14:289-292; Choi and Ljungdahl (1996) Biochemistry 35:4897-4905], and thus Avicel adsorption was used to assess association of the feruloyl activity with cellulosomes. As shown in Table 2, 97.1% of total feruloyl activity was removed from the culture medium by Avicel treatment, even higher than the percentages of cellulase (80.5%) and xylanase (73.3%) activities removed. These data indicate that **feruloyl esterases** produced by C. thermocellum possess cellulose-binding ability through either a cellulose-binding domain or the cellulosomes. XynZ has a family VI cellulose binding domain [Grepinet et al. (1988) supra; Tomme et al. (1995) supra] and a docking domain between the CBD and the dockerin, whereas XynY contains a docking domain.

Detailed Description Text - DETX (10):

Cellulosomes eluted from Avicel adsorption were analyzed by gel filtration chromatography using a Superose 6 column to assess the sizes of proteins containing **feruloyl esterase** activity in the native state. The majority of the proteins were eluted in fractions containing molecules with sizes around 2.0 million daltons (FIG. 3), characteristic of cellulosomes eluted from gel filtration [Choi and Ljungdahl (1996) supra]. **Feruloyl esterase** activity in the fractions correlated well with fractions of cellulosomes. No activity was found in fractions with protein molecules less than 200 kDa, indicating that **feruloyl esterase** activity resides in the cellulosome.

Detailed Description Text - DETX (11):

The UD coding region of XynY and various regions of XynZ were over-expressed in E. coli using the pRSET system (Invitrogen, Carlsbad, Calif.). Constructs spanning the XynY UD sequence, XynZ UD alone, and UD plus the CBD sequence in PRSET gave high levels of **feruloyl esterase** activity whereas cell-free extracts of E. coli harboring the pET-21 b recombinant plasmid failed to hydrolyze FAXX. Constructs with 20 and 40 amino acid residues deleted from the C-terminus of the XynZ UD did not hydrolyze FAXX, indicating that XynZ sequence from the end of the signal peptide up to amino acid 288 was required to form an active **feruloyl esterase**. The heterologous protein band of the UD constructs without IPTG induction on SDS-PAGE analysis reached 40-50% of total protein. Both growth rates and levels of feruloyl activity of the constructs with the XynY and XynZ sequences were lower with IPTG induction than without induction. Without wishing to be bound by theory, it is believed that low level of T7 polymerase in E. coli BL21 (DE3) strain was ideal for the expression of the inserted genes in pRSET B, and over-expression of T7 polymerase gene by IPTG induction resulted in toxic levels of **feruloyl esterase** production.

Detailed Description Text - DETX (12):

Amino acid residues 328 to 419 of XynZ were homologous to two repeated CBDs of C. stercorarium XynA [Sakka et al. (1993) supra; Sakka et al. (1995) supra] (FIG. 4). This domain has been recently classified as a family VI CBD [Tomme et al. (1995) supra]. Constructs containing the UD alone and both the UD plus the putative CBD of XynZ were purified from recombinant E. coli cultures. The majority of **feruloyl esterase** activity of the polypeptide containing both domains was removed by Avicel and acid swollen cellulose adsorption but not with the UD alone, indicating that strong cellulose binding capability resides in the family VI cellulose binding domain of XynZ. Cellulose-binding ability was confirmed with native gel retardation analysis.

Detailed Description Text - DETX (16):

In order to understand how microorganisms breakdown plant cell wall material, we chose to study enzymes from Clostridium thermocellum. In particular, XynY and XynZ from this organism were originally thought to contain a xylanase domain and second domain of unknown function. We have now demonstrated that the function of this domain is that of a **feruloyl esterase,** which is functional in the cellulosome or as a free protein. **Feruloyl esterases** are important for the complete degradation of plant cell wall material. These enzymes are produced by several organisms, but they have not

been found in a bifunctional enzyme.

Detailed Description Text - DETX (17):
A **feruloyl esterase** from Orpinomyces PC-2 was purified and internal fragments of the enzyme were used to screen the Orpinomyces PC-2 cDNA library. A partial clone was sequenced and showed homology to XynZ. A BLAST analysis showed that this enzyme, along with XynY, had domains of unknown function.

Detailed Description Text - DETX (18):
The high temperature stability of the enzyme is surprising because no other thermophilic **feruloyl esterases** have been reported until the present disclosure of the C. thermocellum thermotolerant **feruloyl esterases**. The Orpinomyces PC-2 enzyme has substrate specificity for both feruloyl and p-coumaroyl esterified substrates. The clostridial enzymes are the first from bacteria to have such a dual role. Although the Orpinomyces enzyme is not a true **p-coumaroyl esterase,** no **p-coumaric acid esterases** have been found in bacteria to date.

Detailed Description Text - DETX (20):
Feruloyl esterases and xylanase act synergistically to the release of ferulic acid and reducing sugars from lignocellulosic material [Borneman et al. (1993) supra]. In C. thermocellum XynY and XynZ, we hypothesize that this is more efficient due to the incorporation of both enzymes into one. We believe there is a multicutting event catalyzed by these enzymes much like the multicutting event in the cellulosome itself which leads to more efficient hydrolysis of plant cell wall material. The substrate, arabinoxylan could be passed from one active site to another, which would eliminate the process of each of two enzymes having to bind to the substrate and then release it for the other enzyme to attack.

Detailed Description Text - DETX (21):
XynY and XynZ are enzymatic components of the Clostridium thermocellum cellulosome. These components have a multi-domain structure which includes a dockerin domain, a catalytic xylanase domain, and a domain of unknown function. The previously unknown domains in XynY and XynZ have been found to have **phenolic esterase** activity. These domains have some amino acid homology to that of a **phenolic esterase** from the anaerobic fungus Orpinomyces sp. strain PC-2. Secondly, purified cellulosomes from C. thermocellum hydrolyze (O-[5-O-[(E)-feruloyl]-(-L-arabinofuranosyl]-(1(3)-O-(-D-xylopyranosyl-(1(4)-D-xylopyranose) (FAXX) and [5-O-[(E)-feruloyl]-[O-(-D-xylopyranosyl-(1(2)]-O-(-L-arabinofuranosyl-[1(3]]-O-(-D-xylopyranosyl-(1(4)-D-xylopyranose (FAX.sub.3) yielding ferulic acid as a product, thus indicating the presence of a **phenolic acid esterase**. Intracellular and extracellular fractions lacking cellulosomes had insignificant amounts of **phenolic acid esterase** activity which confirmed that the activity resided with the cellulosome. The final proof was obtained by cloning the domains of XynY and XynZ into Escherichia coli. The domains were expressed and found to possess **phenolic acid esterase** activities with FAXX and FAX.sub.3 as substrates.

Detailed Description Text - DETX (22):

Nucleotides corresponding to regions of DNA encoding amino acids in XynZ (Genbank Accession Number M22624) from 20-421 and in XynY (Genbank Accession Number X83269) from 795-1077 were overexpressed in E. coli using the pET and pRSET systems respectively. The XynZ sequence will henceforth be referred to as XynZ FAE/CBD since it incorporates the family VI CBD, and the XynY protein is XynY FAE since it only contains a catalytic domain. The cell free extracts containing the expressed proteins each hydrolyzed FAXX with release of ferulic acid (FA) which suggests that these proteins are **feruloyl esterases**. The expressed protein from the construct containing XynY FAE had a molecular weight of 31 kDa, consistent with the sequence data. Constructs containing XynZ FAE/CBD produced a protein with a molecular mass of 45 kDa as analyzed by SDS-PAGE. The protein was expressed without IPTG induction at a level of 8% of the total protein. Levels of **feruloyl esterase** activity of the constructs with the XynY FAE and XynZ FAE/CBD sequences were lower with IPTG induction than without induction. Since these proteins had similar sequences and similar function coupled with the fact that XynZ had higher expression levels than XynY, we decided to focus our attention on XynZ and subsequent experiments will refer to that protein.

Detailed Description Text - DETX (23):

Constructs were made which corresponded to proteins with amino acids from the original C. thermocellum XynZ sequence of 20-307 (FAE287), 20-286 (FAE) and 20-247 (FAE227) (with reference to SEQ ID NO:14 and FIG. 2). FAE287 is missing the CBD, but contains a proline rich linker which separates the CBD from the FAE domain while FAE does not contain this linker. When these constructs were expressed in E. coli in the same manner as XynZ FAE/CBD, they both exhibited **feruloyl esterase** activity. Thus, the removal of the 114 amino acids of the CBD did not have a detrimental effect on the activity. XynZ FAE/CBD bound to acid swollen cellulose very weakly, while the other constructs missing the CBD did not bind acid swollen cellulose at all. FAE227 was an inactive but expressed enzyme. Neither the CBD nor the linker is necessary for activity, but amino acids 247-266 are necessary for generation of an active enzyme. Since neither the linker region nor the CBD is necessary for activity, we used the smallest construct which still retained activity, FAE, for subsequent experiments.

Detailed Description Text - DETX (24):

The XynZ FAE/CBD polypeptide was purified from E. coli cell free extract after a single step of heat treatment at 70.degree. C. for 30 min. Over 200 mg of the XynZ FAE/CBD were obtained from 2.5 gram of crude protein (Table 3). The purified XynZ FAE/CBD had a mass as stated previously of 45 kDa as revealed by SDS-PAGE (FIG. 5), consistent with the calculated size (46.5 kDa). There was no evidence for aggregation of the **feruloyl esterase** produced in E. coli, and SDS-PAGE gels showed that protein which was removed from the cell free extract by centrifugation had no insoluble protein which could be attributed to inclusion bodies.

Detailed Description Text - DETX (27):

Anaerobic microorganisms do not readily degrade lignin, but are able to solubilize it. Anaerobic fungi are able to solubilize but not metabolize lignin, and it is suggested that the released lignin was carbohydrate linked [McSweeney et al. (1994) Appl. Environ. Microbiol. 60:2985-2989]. The data herein indicate that **feruloyl esterases** are responsible for lignin solubilization. Most studies of the cellulosome of C. thermocellum has been directed toward its celluloytic activity. It also has xylanases which we have shown are bifunctional enzymes with **feruloyl esterase** activity. The cellulosome should be efficient in the degradation of arabinoxylan. It has been previously shown that Clostridium xylanolyticum released aromatics into the culture medium when grown on lignocellulosic material [Rogers et al. (1992) International Biodeterioration & Biodegradation 29:3-17].

Detailed Description Text - DETX (28):

XynY and XynZ each contain a glycosyl hydrolase family 10 catalytic domain in addition to the FAE catalytic domain. The xylanase domain of XynZ has been well studied, that construct has been crystallized, and the three dimensional structure solved [Dominguez et al. (1995) Nat. Struct. Biol. 2:569-576; Souchon et al. (1994) J. Mol. Biol. 235:1348-1350]. In general, xylanases are thought to be sterically hindered by groups substituted on the xylan backbone. **Feruloyl esterase** and xylanase have been shown to act synergistically for the release of ferulic acid and reducing sugars from lignocellulosic material [Borneman et al. (1993) supra]. In XynY and XynZ we hypothesize that this event has been made more efficient by the incorporation of both FAE and xylanase catalytic domains into one enzyme. Without wishing to be bound by theory, we believe that there is a multicutting event catalyzed by these enzymes much like the multicutting event in the cellulosome itself which leads to more efficient hydrolysis of plant cell wall material. Bifunctional enzymes like XynY and XynZ form a dumbbell-like shape which attacks the arabinoxylan polysaccharide and the substrate is passed from one active site to another, eliminating the relatively inefficient two enzyme process in which one has to bind to the substrate and then release it for the other enzyme to attack. The existence of multidomain enzymes such as the sea whip coral peroxidase-lipoxygenase [Koljak et al. (1997) Science 277:1994-1996] and a xylanase-.beta.(1,3-1,4)-glucanase from Ruminococcus flavifaciens [Flint et al. (1993) J. Bacteriol. 175:2943-2951] suggests an evolutionary importance of having two or more catalytic domains in one enzyme. XynZ contains a contains a family VI CBD, which does not bind cellulose significantly. However, representatives of CBDs of this family usually efficiently bind xylan. The CBD of XynZ may participate in a tight association of the catalytic domains with the substrate. This is consistent with the higher Km of FAE as compared to that of XynZ FAE/CBD.

Detailed Description Text - DETX (30):

The FAE domains of XynZ and XynY are homologous to each other and to the Orpinomyces FaeA. The Orpinomyces FaeA, together with those homologous sequences, however, failed to show significant homology to the recently published **feruloyl esterases** (FaeA) of Aspergillus niger and A. tubingensis [de Vries et al. (1997) supra] as well as CinA and CinB from Butyrivibrio fibrisolvens [Dalrymple et al. (1996) FEMS Microbiol. Lett. 143:115-120; Dalrymple and Swadling (1997) Microbiology 143:1203-1210] and XylD from

Pseudomonas fluorescens subsp. cellulosa [Ferreira et al. (1993) Biochemical Journal 294:349-355]. The sequence analysis implies that a new type of **feruloyl esterase** is encoded by the Orpinomyces gene and the homologous C. thermocellum sequences described above. The Orpinomyces FaeA, and the FAE domains of XynZ and XynY were also shown to be homologous to a hypothetical polypeptide of E. coli (Genbank Accession Number P31471) and the carboxy terminal region of a Ruminococcus sp. xylanase earlier designated as a UD [Genbank Accession Number S58235]. No function had been assigned to those sequences of E. coli and Ruminococcus. Without wishing to be bound by theory, the present inventors believe that these sequences also encode **feruloyl esterases** and that the Ruminococcus xylanase is also bifunctional. Ruminococcus has been shown to produce FAE activity [McSweeney et al. (1998) Anaerobe 4:57-65], and another Ruminococcus xylanase has been shown to be a bifunctional enzyme with xylanase and acetyl xylan esterase activity [Kirby et al. (1998) Biochemical Society Transactions 26:S169]. No **feruloyl esterase** activity has been observed in E. coli. The gene from E. coli may encode a dipeptidase instead, because homology exists between a dipeptidase from Aspergillus fumigatus and **feruloyl esterases**. The data suggest a common ancestral gene encoding **feruloyl esterases** from Orpinomyces, C. thermocellum, and Ruminococcus.

Detailed Description Text - DETX (31):

Applications for the **phenolic acid esterase** enzymes of the present invention, especially the **feruloyl esterases**, include producing ferulic acid from wheat bran or agricultural byproducts, using the enzyme to treat grasses, grains or other plant materials which are used in the pulp and paper industry, feed processing, and as a food additive. These thermostable enzymes have advantages over other enzymes because they are easy to purify, have high temperature optima and are stable over a wide pH range.

Detailed Description Text - DETX (32):

Any of the **feruloyl esterases** as described (see also U.S. patent application Ser. No. 09/390,224, filed Sep. 3, 1999, incorporated by reference herein) can be used as food or feed supplements. Other phenolic acid or **feruloyl esterases** known to the art can also be used in the methods and compositions of the present invention. For example, esterases isolated after production from a naturally occurring microbial strain or through recombinant production using nucleic acids ultimately derived from a naturally occurring strain are contemplated. Naturally occurring microbes which produce **phenolic acid esterases** and/or **feruloyl esterases** include, without limitation, strains of Aureobasidium, Trichoderma, Aspergillus, Bacillus, Streptomyces, Penicillium, Neocallimastix, and Humicola, as well known in the art.

Detailed Description Text - DETX (34):

When a grain or other plant-derived food or feed component having a substantial non-starch polysaccharide content is used, the energy source availability can be increased by treatment with a **feruloyl esterase** and a xylanase at a ration of 1 to 200 U/kg for each enzyme, desirably about 10 to about 50 U/kg feed or food. As shown in Table 12, there appears to be a

synergism between xylanase and **feruloyl esterase**.

Detailed Description Text - DETX (35):

Food or feed can be supplemented or treated with the **feruloyl esterase** and xylanase to improve nutrition and energy source availability for humans, poultry (e.g., chickens, turkeys, ducks, geese, and other fowl), swine, sheep, cattle, horse, goats, fish (including but not limited to salmon, catfish, tilapia and trout) and shellfish, especially shrimp, and other farmed animals.

Detailed Description Text - DETX (36):

Food or feed ingredients which are improved by treatment with **feruloyl esterase** and xylanase include, without limitation, wheat, rye, barley, oats, corn, rice, soybean, millet, sorghum, grasses, legumes and other pasture and forage plants. Fresh or dry feed or food components can be treated with a liquid comprising the xylanase and **phenolic acid esterase** so that the particles of the food or feed are coated with the enzymes. Similarly, wet or dry enzyme compositions can be added to a liquid food or feed composition so that the ratio of enzymes to dry weight or plant material is as taught herein.

Detailed Description Text - DETX (37):

The present inventors have demonstrated the usefulness of **feruloyl esterase** as an animal feed additive, as described in Example 7 below. Wheat represents a potential energy source in poultry and swine or other rations, for example, but it is frequently avoided because of its low energy value relative to corn. The lower energy availability is due to the presence of a significant amount of non-digestible fiber or non-starch polysaccharide (NSP). In addition to NSP being unavailable for energy, it also acts as an anti-nutritional factor and reduces digestibility of other components of the diet. The availability of fiber-degrading enzymes that can be added to wheat diets has increased interest in the use of wheat and other grains for poultry and swine rations. Three day old broiler chicks were fed an energy-deficient diet or the same diet supplemented with Orpinomyces or other **feruloyl esterase** protein, a xylanase A (for example from Orpinomyces or from Aureobasidium pullulans for example [see U.S. Pat. No. 5,591,619, Li et al., 1997]) or a combination of these enzymes. See Table 11 for composition of the basal diet and Example 7. The results of this feeding experiment are summarized in Table 12. Weight gain and feed efficiency in the control group is set to 100. **Feruloyl esterase** alone did not significantly affect weight gain. Birds grew slightly slower (97% of control) and required slightly more feed per unit of gain than the control, unsupplemented group. Xylanase alone improved growth rate 7% and feed efficiency 5%. The combination of xylanase and **feruloyl esterase** showed the greatest response, with a 15% improvement in growth rate and a 10% improvement in efficiency over that in the control group.

Detailed Description Text - DETX (38):

Besides promoting increased nutrient availability, **feruloyl esterase** can be used to improve the ferulic acid availability in a food or feed. Ferulic acid has antioxidant activity, and it can be made available through **feruloyl**

esterase treatment of a foodstuff. Ferulic acid is an antioxidant, and accordingly, there is interest in its use to promote general health, to act as an anti-tumor agent and as an anti-aging agent. For example, wheat can be treated with **feruloyl esterase,** advantageously in combination with xylanase, and be consumed to serve as a ferulic acid supplement, especially in humans. Its consumption then improves the oxidant/antioxidant status and the general health of the consumer.

Detailed Description Text - DETX (39):

In addition to plant-derived solid food or feed treated with **feruloyl esterase or the combination of feruloyl esterase** and xylanase, liquids (beverages, e.g.) can also comprise **feruloyl esterase (or feruloyl esterase** and xylanase) treated material or soluble products thereof. If the beverage contains the solid foodstuff or feedstuff, enzymes(s) is(are) added at a ratio of from about 1 to 200 units of enzyme per kg, desirably from about 10 to about 50 U/kg of esterase and for xylanase, from about 100 to about 10,000 U/kg dry weight of plant-derived material in the liquid foodstuff or beverage.

Detailed Description Text - DETX (40):

A **feruloyl esterase** described herein, desirably in combination with a cellulase and/or xylanase, for example that from Orpinomyces PC-2, can also be used in the pulping and paper recycling industries. The ratio of the esterase to solids is from about 0.1 to about 200 U/kg dry weight, desirably from about 1 to about 100 U/kg, and advantageously from about 10 to about 50 U/kg.

Detailed Description Text - DETX (41):

The **feruloyl esterase or combination of feruloyl esterase** and xylanase can be formulated as dry materials or as liquid concentrates for subsequent use in combination with a source of plant-derived non-starch polysaccharide or poorly digestible plant fiber material to be treated. Such a formulation can be freeze-dried in the case of a dry material or it can be a liquid concentrate. A liquid formulation can contain from about 100 ug. to about 50 mg/ml of protein. Reducing agents such as cystine dithiothreitol, dethioerythritol or .beta.-mercaptoethanol can be included to prevent enzyme oxidation, and protein stabilizing agents, for example glycerol (0.1% to 10% w/v), sucrose (0.1% to 10% w/v) among others, can be included, or an irrelevant protein such as bovine serum albumin or gelatin, can also be present. Although the esterases of the present invention are stable, a buffering agent can be added to stabilized the pH in the range of about 4.5 to 7.8.

Detailed Description Text - DETX (42):

The **feruloyl esterase** domain of XynZ was highly expressed in E. coli and the esterase comprised 40-50% of the total cell protein. The recombinant esterase of XynZ was purified to almost homogeneity by heat treatment. The protein had a molecular mass of 45 kDa, consistent with the size of the predicted deduced amino acid sequence. Of the substrates tested, the expressed protein had high specific activity towards FAXX and FAX.sub.3. With FAX.sub.3 as a substrate Km and Vmax values were 3.2 mM and 13.5 .mu.mol ferulic acid released min-1 mg-1 respectively at pH 6.0 at 60.degree. C. Several phenolic esterified substrates

were hydrolyzed and the specific activities with those containing feruloyl groups were higher than were those with p-coumaroyl groups confirming that the previously unknown domain of XynZ is a **feruloyl esterase**. The enzyme released mainly ferulic acid from wheat bran and Coastal Bermuda grass (CBG) with a smaller amount of p-coumaroyl groups released from CBG. This study represents the first demonstration of esterases in the cellulosome of Clostridium thermocellum and of enzymes from the cellulosome with two different activities. The present work also provides a **phenolic acid esterase** derived from a xylanase from Ruminococcus and as an enzyme produced by Orpinomyces PC-2.

Detailed Description Text - DETX (43):

A summary of the purification of FAE from Orpinomyces sp stain PC-2 is presented in Table 7. The Q-Sepharose column separated two peaks of esterase activity. Proteins which eluted in the first peak had higher activity against ethyl-pCA while proteins eluting in the second peak had greater activity against FAXX. These data suggest that a **p-coumaroyl esterase** eluted in the first peak while the **feruloyl esterase** eluted in the second. The first peak was not studied further, but the fractions in peak 2 were further purified resulting in a purified enzyme which had an approximate molecular mass of 50 kDa as visualized by SDS-PAGE analysis (FIG. 7). There was a decrease in specific activity after the MonoQ step which could not be explained.

Detailed Description Text - DETX (45):

Two of the peptide fragments from the internal amino acid sequencing were used to create degenerative olignucleotide primers which are listed in the materials and methods section. These primers were used to amplify regions of DNA in the Orpinomyces PC-2 cDNA library. A 216 bp PCR product was generated. The PCR product was labeled with digoxygenin-UTP and used as a probe to screen the cDNA library. After screening 50,000 phage, one positive plaque was obtained and its DNA was sequenced using T3 and T7 universal primers. Sequencing using the T3 primer did not reveal any ORFs, however, sequencing using the T7 reverse primer gave the C-terminal end of the gene. Based on the sequence data and restriction fragment analyses, but without wishing to be bound by theory, we have concluded that the faeA gene in this cDNA was truncated and furthermore that the insert comprises multiple genes. These other genes were not studied further. The deduced amino acid sequence of the insert matched the data from the peptide sequencing. The insert had a size of 1074 bp and encoded a protein of 358 amino acids. Since the size of the encoded protein did not match that of the purified enzyme and the N-terminal sequence, including a signal peptide and lack of a start codon, another round of screening was performed using the entire sequence as a probe after digoxygenin labeling. After screening an additional 50,000 phage, one positive clone was obtained which had a size of 1673 bp with the largest open reading frame comprising a protein of 530 amino acids. The sequence of this insert is believed to be an incomplete one since no 5' UTR was found and the (putative) signal sequence has only four amino acids. Most signal sequences found in hydrolytic enzymes from anaerobic fungi are at least 20 amino acids long. The insert was found to be in a reverse orientation with respect to the lacZ promoter. The upstream lac promoter should direct synthesis of the inserted gene, but no activity was found in lysed E. coli cells harboring the recombinant plasmid. The faeA gene in E. coli was expressed using the pET

system (Novagen) in the correct orientation. The recombinant FaeA released ferulic acid from FAXX as well as other substrates which were esterified with phenolic groups. The enzyme had the highest activity against FAXX, which demonstrates that it is a true **feruloyl esterase** (Table 10). In addition, when the enzyme was incubated with a recombinant xylanase, there was a 80 fold increase in FA released over FaeA alone.

Detailed Description Text - DETX (47):

It will be understood by those skilled in the art that other nucleic acid sequences besides those disclosed herein for the **phenolic acid esterases, i.e. feruloyl esterases,** will fiction as coding sequences synonymous with the exemplified coding sequences. Nucleic acid sequences are synonymous if the amino acid sequences encoded by those nucleic acid sequences are the same. The degeneracy of the genetic code is well known to the art. For many amino acids, there is more than one nucleotide triplet which serves as the codon for a particular amino acid, and one of ordinary skill in the art understands nucleotide or codon substitutions which do not affect the amino acid(s) encoded. It is further understood in the art that codon substitutions to conform to common codon usage in a particular recombinant host cell is sometimes desirable.

Detailed Description Text - DETX (48):

Specifically included in this invention are sequences from other strains of Clostridium and from other microorganisms which hybridize to the sequences disclosed for **feruloyl and coumaryl esterases** under stringent conditions. Stringent conditions refer to conditions understood in the art for a given probe length and nucleotide composition and capable of hybridizing under stringent conditions means annealing to a subject nucleotide sequence, or its complementary strand, under standard conditions (i.e., high temperature and/or low salt content) which tend to disfavor annealing of unrelated sequences, (indicating about 95-100% nucleotide sequence identity). Also specifically included in this invention are sequences from other strains of Orpinomyces species and other anaerobic fungi which hybridize to the sequences disclosed for the esterase sequences under moderately stringent conditions. Moderately stringent conditions refer to conditions understood in the art for a given probe sequence and "conditions of medium stringency" means hybridization and wash conditions of 50.degree.-65.degree. C., 1.times.SSC and 0.1% SDS (indicating about 80-95% similarity). Also specifically included in this invention are sequences from other strains of Orpinomyces, from other anaerobic fungi, and from other organisms, including bacteria, which hybridize to the sequences disclosed for the esterase sequences under highly stringent conditions. Highly stringent conditions refer to conditions understood in the art for a given probe sequence and "conditions of high stringency" means hybridization and wash conditions of 65.degree.-68.degree. C., 0.1.times.SSC and 0.1% SDS (indicating about 95-100% similarity). Hybridization assays and conditions are further described in Sambrook et al. (1989).

Detailed Description Text - DETX (49):

A method for identifying other nucleic acids encoding **feruloyl esterase**- and/or coumaryl esterase-homologous enzymes is also provided wherein nucleic

acid molecules encoding **phenolic acid esterases** are isolated from an anaerobic fungus, including but not limited to Orpinomyces or an anaerobic bacterium, such as Clostridium or Ruminococcus, among others, and nucleic acid hybridization is performed with the nucleic acid molecules and a labeled probe having a nucleotide sequence that includes all or part of a FAE coding sequence as given in Table 5, 6, 9 and/or 10 herein. By this method, **phenolic acid esterase** genes similar to the exemplified **feruloyl and coumaryl esterases** can be identified and isolated from other strains of Clostridium or other anaerobic microorganisms. All or part of a nucleotide sequence refers specifically to all continuous nucleotides of a nucleotide sequence, or e.g. 1000 continuous nucleotides, 500 continuous nucleotides, 100 continuous nucleotides, 25 continuous nucleotides, and 15 continuous nucleotides.

Detailed Description Text - DETX (50):

Sequences included in this invention are those amino acid sequences which are 40 to 100% identical to the amino acid sequences encoded by the exemplified C. thermocellum strain **feruloyl esterase,** amino acids proteins truncated from the XynY or XynZ proteins or the Ruminococcus FAE polypeptide or the Orpinomyces PC-2 FAE polypeptide, all specifically identified herein. Sequences included in this invention are also those amino acid sequences which are 40, 50, 60, 70, 75, 80, 85, 90, 95 to 100%, and all integers between 40% and 100%, identical to the amino acid sequences encoded by an exemplified **phenolic acid esterase** coding sequence and corresponding to or identifying encoded proteins which exhibit **feruloyl esterase** activity. In comparisons of protein or nucleic acid sequences, gaps introduced into either query or reference sequence to optimize alignment are treated as mismatches. In amino acid sequence comparisons to identify **feruloyl esterase** proteins, the reference sequence is, desirably, amino acids 227 to 440 of SEQ ID NO:18 (FAE of Orpinomyces PC-2).

Detailed Description Text - DETX (53):

Techniques for genetically engineering plant cells and/or tissue with an expression cassette comprising an inducible promoter or chimeric promoter fused to a heterologous coding sequence and a transcription termination sequence are to be introduced into the plant cell or tissue by Agrobacterium-mediated transformation, electroporation, microinjection, particle bombardment or other techniques known to the art. The expression cassette advantageously further contains a marker allowing selection of the heterologous DNA in the plant cell, e.g., a gene carrying resistance to an antibiotic such as kanamycin, hygromycin, gentamicin, or bleomycin. Assays for **phenolic acid esterase** and/or xylanase enzyme production are taught herein or in U.S. Pat. No. 5,824,533, for example, and other assays are available to the art.

Detailed Description Text - DETX (60):

Monoclonal or polyclonal antibodies, preferably monoclonal, specifically reacting with the **phenolic acid esterases** of the present invention may be made by methods known in the art. See, e.g., Harlow and Lane (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratories; Goding (1986) Monoclonal Antibodies: Principles and Practice, 2d ed., Academic Press, New York.

Detailed Description Text - DETX (70):

Genomic DNA was isolated from C. thermocellum as previously described [Maniatis et al. (1982) supra]. PCR primers were designed (Table 1) and synthesized on an Applied Biosystems (Foster City, Calif.) DNA sequencer. To facilitate the insertion of DNA sequence into or pET-21 b or pRSET B, BamHI (for pET-216) or NdeI for pRSET B, and HihdIII sites were added to forward and reverse primers, respectively (Table 1). PCRs were carried out on a Perkin Elmer 480 Thermocycler for 30 cycles with each cycle on 95.degree. C. for 1 min, 48.degree. C. for 1 min, and 72.degree. C. for 3 min. PCR products and the plasmid were digested with BamHI (or NdeI) and HindIII, purified with a Bio101 Geneclean kit, ligated with T4 ligase. E. coli BL21(DE3) was transformed with the ligation mixture and at least four colonies of each construct were picked for analyzing **feruloyl esterase** expression. The inserted sequences were sequenced to verify the lack of unwanted mutations.

Detailed Description Text - DETX (71):

Two internal sequences were used to create degenerate oligonucleotide primers for PCR in order to amplify the **feruloyl esterase** coding sequence in the cDNA library in Orpinomyces. The Orpinomyces PC-2 cDNA library is described in the .lambda.ZAPII vector (Stratagene, La Jolla, Calif.) in E. coli host cells is described in Chen et al. (1995) Proc. Natl. Acad. Sci. 92:2587-2591. Positive clone(s) are subclonal into a pBluescript vector (Stratagene, La Jolla, Calif.). The amplified product was cloned into pCRII (Invitrogen, Carlsbad, Calif.) using the TA cloning kit and sequenced using an automatic PCR sequencer (Applied Biosystems, Foster City, Calif.) using M13 reverse primer. The resulting PCR product was used to screen the cDNA library after being labeled with digoxigenin (Boehringer Mannheim, Indianapolis, Ind.). The digoxigenin probe was bound to plaques which were lifted from a nitrocellulose blot. Antibodies conjugated to alkaline phosphatase showed a single positive clone which hybridized to the PCR product. The product was sequenced and found to contain the C-terminal 358 amino acids of the enzyme (See Table 9). A second probe which incorporated those 339 amino acids was used as a probe to screen the library in the same manner as before. A second clone was isolated which contained the C-terminal region plus an additional 172 amino acids making a polypeptide of 530 amino acids. Confirmation of the sequence came from N-termninal and internal protein sequence data from the purified enzyme which matched that of the cloned cDNA product. Expression cloning of this coding sequence, which lacks an ATG translation start site, can be achieved by expressing it, in frame, as a fusion protein using any one of a number of fusion protein vectors known to the art or an ATG translation start codon and/or ribosome binding site upstream of the ATG can be added using methodology well known to and readily accessible to the art in an expression vector appropriate to the choice of recombinant host cell.

Detailed Description Text - DETX (74):

The cellulosomes were isolated from 10L of culture fluid after complete substrate exhaustion by the affinity digestion method [Morag et al. (1992) supra]. This preparation was used directly for gel filtration using a Fast Protein Liquid Chromatography (FPLC) system with a Superose 6 column

(Pharmacia, Piscataway, N.J.). Proteins were eluted in 50 mM Tris-HCl, 100 mM NaCl at a flow rate of 0.2 ml/min. Fractions of 0.5 ml were collected and stored at 4.degree. C. for further analysis. Cell extracts were prepared by first growing the organism in the presence of 0.2% cellobiose for 2 days. Cells were then separated by centrifugation, resuspended in 50 mM Tris-HCl buffer, pH 7.5, and sonicated. Culture medium was concentrated to 5 ml using a Millipore concentrator (Millipore, Bedford, Mass.). To adsorb cellulosomes from the medium, 0.5 mg of Avicel was added and the suspension was stirred at 4.degree. C. for 4 hours. Avicel was removed by centrifugation (Avicel-treated medium). All fractions were tested for. Avicelase, xylanase, and **ferulic acid esterase** activities.

Detailed Description Text - DETX (75):

Unless otherwise noted, all C. thermocellum enzyme assays were performed at 60.degree. C. in 50 mM Na-citrate buffer, pH 6.0. One unit of enzyme activity was defined as the amount of enzyme that released 1 .mu.mol of product min-1, and specific activity is given in units per milligram of protein. **Feruloyl esterase** activity was measured using a modified version of the assay described by Borneman et al. [Borneman etal. (1990) Anal. Biochem. 190:129-133]. The appropriately diluted protein sample (25 l) was added to 400 .mu.l of buffer plus 8 mM of substrate. Samples were incubated at 60.degree. C. for 5 min. and the reaction was stopped by adding 25 .mu.l of 20% formic acid. Release of ferulic acid was measured via HPLC using a mobile phase of 10 mM Na-formate pH 3 and 30% (vol/vol) methanol. For routine assays, FAXX and FAX3 purified from wheat bran were used as substrates [Borneman et al. (1990) supra]. Ethyl-ferulate and ethyl-p-coumarate esters were a gift from D. E. Akin (USDA, Athens, Ga.). The hydrolysis of these (10 mM) were determined similarly to that of FAXX, but the HPLC analyses were performed with 50% methanol. HPLC runs were with a Hewlett Packard 1100 Series instrument equipped with an autosampler and diode array detector. Ferulic acid and p-coumaric acid were used as standards. To determine the amount of feruloyl and p-coumaroyl groups released from plant cell walls, wheat bran and Coastal Bermuda grass were ground in a Wiley mill to pass through a 250 .mu.m screen. Plant samples of ten milligram were incubated for one hour in 400 .mu.l of 50 mM Na-citrate buffer pH, 6.0 plus 25 .mu.l of enzyme. After the addition of 25 .mu.l of 20% formic acid to stop the reaction, the samples were centrifuged at 16,000.times.g in a microfuge and then assayed for FA and pCA by HPLC.

Detailed Description Text - DETX (77):

Unless otherwise noted, all Orpinomyces enzyme assays were performed at 40.degree. C. in 50 mM Bis-Tris Propane buffer, pH 6.0. One unit of enzyme activity is defined as the amount that released 1 .mu.mol of product min-1, and specific activity is given in units per milligram of protein. Protein was determined by the method of Bradford [Bradford, M. (1976) Anal. Biochem. 72:248-254]. **Feruloyl esterase** activity was assayed by the method of Borneman et al. [(1990) supra] which involved measuring the release of ferulic acid from FAXX via HPLC using a mobile phase of 10 mM Na-formate pH 3 and 30% (vol/vol) methanol. FAXX was purified from wheat bran as previously described [Borneman et al. (1990) supra]. For assay usingethyl-p-coumarate (ethyl-pCA), the substrate (10 mM) was used with 30% methanol in the same mobile phase. Samples were run on a Hewlett Packard 1100 Series instrument equipped with an

autosampler and diode array detector. Ferulic acid and p-coumaric acid were used as standards. The appropriately diluted protein sample (25 .mu.l) was added to 400 .mu.l of buffer containing 750 .mu.M FAXX. Samples were incubated at 40.degree. C. for 30 min. and the reaction was stopped by adding 25 .mu.l of 20% formic acid. pH optimum assays were carried out in 100 mM citrate phosphate buffer in the range of 2.6-7.0, 100 mM phosphate in the range of pH 5.7-6.3, and 100 mM Tris in the range of pH 7.0-9.0. For temperature optimum determination, purified esterase were incubated for 30 minutes at the appropriate temperature within the range of 200 to 70.degree. C.

Detailed Description Text - DETX (83):

A **feruloyl esterase** was purified from culture supernatant of Orpinomyces sp. strain PC-2 (Barichievicz and Calza medium [Barichievicz and Calza (1990) Appl. Environ. Microbiol. 56:43-48] with 0.2% Avicel as carbon source). The enzyme was obtained from a 60 liter culture of the fungus. The culture was grown under an atmosphere of C0.sub.2 for 6 days. The fungal mycelia were removed by filtration through Miracloth (Calbiochem, San Diego, Calif.) The culture supernatant was concentrated 120 fold using a Pellicon system (Millipore, Bedford, Mass.) and a 10 kDa membrane. The concentrate was loaded onto a Q Sepharose (Pharmacia, Piscataway, N.J.) column equilibrated with 20 mM TrisHCl pH 7.5, and proteins were eluted with a gradient of 1 M NaCl in the same buffer. The active fractions were detected by their ability to release ferulic acid from FAXX as measured by HPLC. The active fractions were combined and ammonium sulfate was added to a concentration of 1.7M. The solution was filtered and then loaded onto a Phenyl Sepharose High Performance Chromatography (Pharmacia) column equilibrated with 20 mM TrisHCl pH 7.5 and 1.7 M ammonium sulfate. The protein was eluted by a negative gradient of buffer without ammonium sulfate. Active fractions were concentrated using a Centricon 10 unit (Amicon, Millipore, Bedford, Mass.) and subsequently applied to a TSK 3000SW column (Tosohaas, Montgomeryville, Pa.) which was equilibrated with 20 mM TrisHCl pH 7.5 and 200 mM NaCl. Fractions with activity were combined and loaded directly onto an anion exchange (MonoQ HR 5/5, Pharmacia, Piscataway, N.J.) column equilibrated with 20 mM TrisHCl pH 7.5. The purified enzyme was eluted using a gradient of 0.5 M NaCl. The purification is'summarized in Table 7.

Detailed Description Text - DETX (95):

Table 9 presents the deduced amino acid sequence and cDNA coding sequence of the mature **phenolic acid esterase** of Orpinomyces PC-2.

Detailed Description Text - DETX (96):

FIG. 1 provides the amino acid sequence for a **phenolic acid esterase (feruloyl esterase)** which corresponds to a previously uncharacterized Ruminococcus xylanase. The sequence of the complete coding sequence of that xylanase is available under Accession No. 558235 (Genbank database) (See Table 9). The coding sequence of the **phenolic acid esterase** polypeptide is nucleotide 2164-2895, exclusive of translation start and stop codons.

Detailed Description Text - DETX (100):

To test whether **feruloyl esterase,** (FaeZ from C. thermocellum), xylanase A from Orpinomyces PC-2 or the combination of the two enzymes improved the availability of nutrients, a feeding experiment was carried out using broiler chicks.

Detailed Description Text - DETX (101):

One-day old broiler chicks (Ross male x Arbor Acres female) were obtained locally (ConAgra, Athens, Ga.) and placed in Petersime battery brooders. Birds were housed 8 per pen, and there were 48 pens in each of two studies. Birds had unlimited access to feed and water. After feeding a complete starter ration (University of Georgia starter corn and soybean meal based diet) for 2 days, birds were switched to the experimental diets. The basal experimental diet consisted of 63% ground wheat, 32% soybean meal and vitamins and minerals. The calculated nutrient composition of this diet was: 22% crude protein, 1.22% lysine, 0.92% sulfur amino acids and 2850 kcal/kg. The diet met the National Research Council requirements for all nutrients except energy. The rationale for designing an energy-deficient diet was that exogenous enzyme addition would result in liberation of carbohydrate from the non-starch polysaccharide component of the diet. In two separate studies the effects of xylanase A (U.S. Pat. No. 5,824,533) at either 1000 or 5000 U/kg with and without **feruloyl esterase** at 5 or 25 U/kg was compared to the basal diet with no enzyme addition. Birds were fed test diets for 14 days.

Detailed Description Paragraph Table - DETL (1):

TABLE 1 Primers used in amplifying various regions of xynY and xynZ of C. thermocellum Name Sequence.sup.a Gene Direction Position.sup.b SEQ ID NO:
XYF1Bam.sup.a1 TAGGATCCCCTGTAGCAGAAAATCCTTC xynY Forward 795-800 1
XYF1.sup.c
TACATATGCCTGTAGCAGAAAATCCTTC xynY Forward 795-800 2 XYR1.sup.c
GAGGAAGCTTTTACATGGAAGAAATATGGAAG xynY Reverse 1071-1077 3 XZF1.sup.d
TACATATGCTTGTCACAATAAGCAGTACA xynZ Forward 20-26 4 XZF1Bam
TAGGATCCCTTGTCACAATAAGCAGTACA xynZ Forward 20-26 5 XZR1.sup.d
GAGGAAGCTTTTAGTTGTTGGCAACGCAATA xynZ Reverse 242-247 6 XZR2.sup.d
GAGGAAGCTTACTTCCACACATTAAAATC xynZ Reverse 261-266 7 XZR3.sup.d
GAGGAAGCTTAGTTTCCATCCCTCGTCAA xynZ Reverse 281-286 8 XZR4.sup.d
GAGGAAGCTTAGTCATAATCTTCCGCTTC xynZ Reverse 302-307 9 XZR5.sup.d
GAGGAAGCTTAAACGCCAAAAGTGAACCAGTC xynZ Reverse 414-421 10 .sup.a Restriction sites NdeI and HindIII are underlined and double-underlined, respectively. .sup.a1 Restriction site BamH1 is underlined. .sup.b Amino acid positions are according to xylanase sequences in the data banks. .sup.c XYF1 or XYF1Bam and XYR1 are the forward and reverse primers used to amplify the **feruloyl esterase** domain from xylY (xynY) of C. thermocellum [see Fontes et al. (1995) supra]. .sup.d XZF1 is the forward primer and XZR1-XZR5 are the reverse primers used in the amplification of the **feruloyl esterase** portion of the xynZ of C. thermocellum.

Detailed Description Paragraph Table - DETL (2):

TABLE 2 Distribution of proteins and hydrolytic activities in C. thermocellum culture grown on Avicel Protein **Feruloyl esterase** Avicelase Xylanase Fraction mg/ml % U/ml % U/ml % U/ml % Cell-associated 0.09 39.1

0.005 2.1 0.001 2.4 0.49 5.3 Cultural medium 0.14 60.9 0.238 97.9 0.04 97.6 8.72 94.7 After Avicel treatment 0.11 47.8 0.002 0.8 0.004 9.7 1.56 16.9 Avicel-bound 0.03 13.2 0.24 97.1 0.033 80.5 6.75 73.3

Detailed Description Paragraph Table - DETL (5):

TABLE 4 Substrate specificity of the **feruloyl, esterase** in C. thermocellum XynZ. Substrate Specific activity (U/mg) FAXX 12.5 FAX.sub.3 11.8 PAX.sub.3 1.4.sup.a Ethyl-FA 0.066 Ethyl-pCA 0.022 CMC 0 PNP-arabinopyranoside 0 PNP-glucopyranoside 0 PNP-xylopyranoside 0 Wheat bran 0.06 Coastal Bermuda grass 0.1 .sup.a Calculated value based on substrate concentration used in the assay

Detailed Description Paragraph Table - DETL (8):

TABLE 7 Purification of a **Feruloyl Esterase** from Orpinomyces PC-2 Culture Supernatant Total Total Specific Activity Protein Activity Purification Step (U) (mg) (Umg.sup.-1) Fold Culture Supernatant 32.38 5,830 5.6E-3 1 Concentrate 7.9 1460 5.42E-3 0.96 Q Sepharose 2.58 181 1.43e-2 2.55 Phenyl 1.68 28.2 5.96E-2 10.6 Sepharose HP TSK 3000SW 0.85 0.62 1.39 253 Mono Q HR 5/5 0.26 0.24 1.087 198

Detailed Description Paragraph Table - DETL (13):

TABLE 12 Improvement in Performance Expressed as Percent Relative to Control Feruloyl Xylanase Xylanase A+ Control **Esterase A Feruloyl Esterase** Z Gain 100 97 107 115 Feed Efficiency 100 104 95 90

Detailed Description Paragraph Table - DETL (20):

Val Pro Asp Glu Gly Val Ala Ala Arg Leu Asn Asp Pro Ala Ala Ile 325 330 335 Asn Gln Gln Leu Arg Asn Phe Thr Val Val Val Gly Asp Lys Asp Val 340 345 350 Val Thr Gly Lys Asp Ile Ala Gly Leu Lys Thr Glu Leu Glu Gln Lys 355 360 365 Lys Ile Asn Phe Asp Tyr Gln Glu Tyr Pro Gly Leu Asn His Glu Met 370 375 380 Asp Val Trp Arg Pro Ala Tyr Ala Ala Phe Val Gln Lys Leu Phe Lys 385 390 395 400 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 20 <211> LENGTH: 721 <212> TYPE: PRT <213> ORGANISM: Aspergillus fumigatus <400> SEQUENCE: 20 Met Gly Ala Phe Arg Trp Leu Ser Ile Ala Ala Ala Ala Ser Thr Ala 1 5 10 15 Leu Ala Leu Thr Pro Glu Gln Leu Ile Thr Ala Pro Arg Arg Ser Glu 20 25 30 Ala Ile Pro Asp Pro Ser Gly Lys Val Ala Val Phe Ser Thr Ser Gln 35 40 45 Tyr Ser Phe Glu Thr His Lys Arg Thr Ser Trp Trp Ser Leu Leu Asp 50 55 60 Leu Lys Thr Gly Gln Thr Lys Val Leu Thr Asn Asp Ser Ser Val Ser 65 70 75 80 Glu Ile Val Trp Leu Ser Asp Asp Ser Ile Leu Tyr Val Asn Ser Thr 85 90 95 Asn Ala Asp Ile Pro Gly Gly Val Glu Leu Trp Val Thr Gln Ala Ser 100 105 110 Ser Phe Ala Lys Gly Tyr Lys Ala Ala Ser Leu Pro Ala Ser Phe Ser 115 120 125 Gly Leu Lys Ala Ala Lys Thr Lys Ser Gly Asp Ile Arg Phe Val Ala 130 135 140 Tyr Gly Gln Ser Tyr Pro Asn Gly Thr Ala Tyr Asn Glu Glu Leu Ala 145 150 155 160 Thr Ala Pro Leu Ser Ser Ala Arg Ile Tyr Asp Ser Ile Tyr Val Arg 165 170 175 His Trp Asp Tyr Trp Leu Ser Thr Thr Phe Asn Ala Val Phe Ser Gly 180 185 190 Thr Leu Lys Lys Gly His Gly Lys Asn Gly Tyr Ser Leu Asp Gly Glu 195 200 205 Leu Lys Asn Leu Val Ser Pro Val Lys Asn Ala Glu Ser Pro Tyr Pro 210 215 220 Pro Phe Gly Gly Ala Ser Asp Tyr Asp Leu Ser Pro Asp Gly Lys Trp 225 230 235 240 Val Ala Phe Lys Ser Lys Ala Pro Glu Leu

Pro Lys Ala Asn Phe Thr 245 250 255 Thr Ser Tyr Ile Tyr Leu Val Pro His Asp
Ala Ser Glu Thr Ala Arg 260 265 270 Pro Ile Asn Gly Pro Asp Ser Pro Gly Thr
Pro Lys Gly Ile Lys Gly 275 280 285 Asp Ser Ser Ser Pro Val Phe Ser Pro Asn
Gly Asp Lys Leu Ala Tyr 290 295 300 Phe Gln Met Arg Asp Glu Thr Tyr Glu Ser
Asp Arg Ala Leu Leu Tyr 305 310 315 320 Val Tyr Ser Leu Gly Ser Lys Lys Thr
Ile Pro Ser Val Ala Gly Asp 325 330 335 Trp Asp Arg Ser Pro Asp Ser Val Lys
Trp Thr Pro Asp Gly Lys Thr 340 345 350 Leu Ile Val Gly Ser Glu Asp Leu Gly
Arg Thr Arg Leu Phe Ser Leu 355 360 365 Pro Ala Asn Ala Lys Asp Asp Tyr Lys
Pro Lys Asn Phe Thr Asp Gly 370 375 380 Gly Ser Val Ser Ala Tyr Tyr Phe Leu
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Ala Ser Ala Asn Glu Ile Asp Pro 420 425 430 Glu Leu Lys Gly Leu Gly Pro Ser
Asp Ile Ser Glu Phe Tyr Phe Gln 435 440 445 Gly Asn Phe Thr Asp Ile His Ala
Trp Val Ile Tyr Pro Glu Asn Phe 450 455 460 Asp Lys Ser Lys Lys Tyr Pro Leu
Ile Phe Phe Ile His Gly Gly Pro 465 470 475 480 Gln Gly Asn Trp Ala Asp Gly
Trp Ser Thr Arg Trp Asn Pro Lys Ala 485 490 495 Trp Ala Asp Gln Gly Tyr Val
Val Val Ala Pro Asn Pro Thr Gly Ser 500 505 510 Thr Gly Phe Gly Gln Ala Leu
Thr Thr Ala Ile Gln Asn Asn Trp Gly 515 520 525 Gly Ala Pro Tyr Asp Asp Leu
Val Lys Cys Trp Glu Tyr Val His Glu 530 535 540 Asn Leu Asp Tyr Val Asp Thr
Asp His Gly Val Ala Ala Gly Ala Ser 545 550 555 560 Tyr Gly Gly Phe Met Ile
Asn Trp Ile Gln Gly Ser Pro Leu Gly Arg 565 570 575 Lys Phe Lys Ala Leu Val
Ser His Asp Gly Thr Phe Val Ala Asp Ala 580 585 590 Lys Val Ser Thr Glu Glu
Leu Trp Phe Met Gln Arg Glu Phe Asn Gly 595 600 605 Thr Phe Trp Asp Ala Arg
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Phe Ala Thr Pro Met Leu Val Ile His Ser 625 630 635 640 Asp Lys Asp Tyr Arg
Leu Pro Val Ala Glu Gly Leu Ser Leu Phe Asn 645 650 655 Val Leu Gln Glu Arg
Gly Val Pro Ser Arg Phe Leu Asn Phe Pro Asp 660 665 670 Glu Asn His Trp Val
Val Asn Pro Glu Asn Ser Leu Val Trp His Gln 675 680 685 Gln Ala Leu Gly Trp
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Lys Glu Lys Phe Thr 1 5 10 15 Val Phe Lys Asp 20 <200> SEQUENCE
CHARACTERISTICS: <210> SEQ ID NO 22 <211> LENGTH: 400
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1 5 10 15 Ala Ala Leu Thr Leu Ala Ile Ala Ser Gly Ile Ser Ala Gln Trp Ala 20
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45 Gln Tyr Val Thr Gln Val Asn Ala Asp Asn Ser Val Thr Phe Arg Tyr 50 55 60
Phe Ala Pro Gly Ala Lys Asn Val Ser Val Val Val Gly Val Pro Val 65 70 75 80
Pro Asp Asn Ile His Pro Met Thr Lys Asp Glu Ala Gly Val Trp Ser 85 90 95 Trp
Arg Thr Pro Ile Leu Lys Gly Asn Leu Tyr Glu Tyr Phe Phe Asn 100 105 110 Val
Asp Gly Val Arg Ser Ile Asp Thr Gly Thr Ala Met Thr Asn Pro 115 120 125 Gln
Arg Gln Val Asn Ser Ser Met Ile Leu Val Pro Gly Ser Tyr Leu 130 135 140 Asp
Thr Arg Ser Val Ala His Gly Asp Leu Ile Ala Ile Thr Tyr His 145 150 155 160
Ser Asn Ala Leu Gln Ser Glu Arg Gln Met Tyr Val Trp Thr Pro Pro 165 170 175
Gly Tyr Thr Gly Met Gly Glu Pro Leu Pro Val Leu Tyr Phe Tyr His 180 185 190
Gly Phe Gly Asp Thr Gly Arg Ser Ala Ile Asp Gln Gly Arg Ile Pro 195 200 205
Gln Ile Met Asp Asn Leu Leu Ala Glu Gly Lys Ile Lys Pro Met Leu 210 215 220
Val Val Ile Pro Asp Thr Glu Thr Asp Ala Lys Gly Ile Ile Pro Glu 225 230 235

240 Asp Phe Val Pro Gln Glu Arg Arg Lys Val Phe Tyr Pro Leu Asn Ala 245 250
255 Lys Ala Ala Asp Arg Glu Leu Met Asn Asp Ile Ile Pro Leu Ile Ser 260 265
270 Lys Arg Phe Asn Val Arg Lys Asp Ala Asp Gly Arg Ala Leu Ala Gly 275 280
285 Leu Ser Gln Gly Gly Tyr Gln Ala Leu Val Ser Gly Met Asn His Leu 290 295
300 Glu Ser Phe Gly Trp Leu Ala Thr Phe Ser Gly Val Thr Thr Thr Thr 305 310
315 320 Val Pro Asp Glu Gly Val Ala Ala Arg Leu Asn Asp Pro Ala Ala Ile 325
330 335 Asn Gln Gln Leu Arg Asn Phe Thr Val Val Val Gly Asp Lys Asp Val 340
345 350 Val Thr Gly Lys Asp Ile Ala Gly Leu Lys Thr Glu Leu Glu Gln Lys 355
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Lys Lys Met Ala Ala Met 1 5 10 gca acg agt ata att atg gct atc atg atc atc
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Ser Ile Pro 15 20 25 gta ctc gcc ggg cga ata att tac gac aat gag aca ggc aca
cat gga 568 Val Leu Ala Gly Arg Ile Ile Tyr Asp Asn Glu Thr Gly Thr His Gly
30 35 40 ggc tac gac tat gag ctc tgg aaa gac tac gga aat acg att atg gaa 616
Gly Tyr Asp Tyr Glu Leu Trp Lys Asp Tyr Gly Asn Thr Ile Met Glu 45 50 55 ctt
aac gac ggt ggt act ttt agt tgt caa tgg agt aat atc ggt aat 664 Leu Asn Asp
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aga aaa ggg aga aaa ttt aat tcc gac aaa acc tat caa 712 Ala Leu Phe Arg Lys
Gly Arg Lys Phe Asn Ser Asp Lys Thr Tyr Gln 80 85 90 gaa tta gga gac ata gta
gtt gaa tat ggc tgt gat tac aat cca aac 760 Glu Leu Gly Asp Ile Val Val Glu
Tyr Gly Cys Asp Tyr Asn Pro Asn 95 100 105 gga aat tcc tat ttg tgt gtt tac
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Asn Val Tyr Lys Asn Glu Ile Arg Ile Gly Ala Asn 220 225 230 235 cca act cct
gcc cca tct caa agc cca att aga aga gat gca ttt tca 1192 Pro Thr Pro Ala Pro
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acc tca att caa atc 1384

Claims Text - CLTX (1):
1. A method for increasing free ferulic acid content of a plant-derived composition, said method comprising the step of contacting a plant-derived composition with a **phenolic acid esterase wherein said phenolic acid esterase** comprises the amino acid sequence of SEQ ID NO:18, amino acids 1 to 530 or wherein the **phenolic acid esterase** consists of an amino acid sequence selected from the group consisting of amino acids 795 to 1077 of SEQ ID NO:12, amino acids 546 to 789 of SEQ ID NO:16, amino acids 20 to 286 of SEQ ID NO:14, amino acids 20 to 307 of SEQ ID NO:14, and amino acids 20 to 421 of SEQ ID NO:14.

Claims Text - CLTX (2):
2. The method of claim 1, wherein said **phenolic acid esterase is a feruloyl esterase**.

Claims Text - CLTX (3):
3. The method of claim 2, wherein the **feruloyl esterase** comprises the amino acid sequence of SEQ ID NO:18, amino acids 1 to 530.

Claims Text - CLTX (4):
4. The method of claim 2, wherein the **feruloyl esterase** consists of the amino acid sequence of SEQ ID NO:12, amino acids 795 to 1077.

Claims Text - CLTX (5):
5. The method of claim 2, wherein the **feruloyl esterase** consists of the amino acid sequence of SEQ ID NO:16, amino acids 546 to 789.

Claims Text - CLTX (6):
6. The method of claim 2, wherein the **feruloyl esterase** consists of the amino acid sequence of SEQ ID NO:14, amino acids 20 to 286.

Claims Text - CLTX (7):
7. The method of claim 2, wherein the **feruloyl esterase** consists of the amino acid sequence of SEQ ID NO:14, amino acids 20 to 307.

Claims Text - CLTX (8):
8. The method of claim 2, wherein the **feruloyl esterase** consists of the amino acid sequence of SEQ ID NO:14, amino acids 20 to 421.

Claims Text - CLTX (14):
14. The method of claim 13, wherein the **phenolic acid esterase** comprises the amino acid sequence of SEQ ID NO:18, amino acids 1 to 530.

Claims Text - CLTX (15):
15. The method of claim 9, wherein the **phenolic acid esterase** consists of the amino acid sequence of SEQ ID NO:12, amino acids 795 to 1077.

Claims Text - CLTX (16):
16. The method of claim 9, wherein the **phenolic acid esterase** consists of the amino acid sequence of SEQ ID NO:16, amino acids 546 to 789.

Claims Text - CLTX (17):
17. The method of claim 9, wherein the **phenolic acid esterase** consists of the amino acid sequence of SEQ ID NO:14, amino acids 20 to 286.

Claims Text - CLTX (18):
18. The method of claim 9, wherein the **phenolic acid esterase** consists of the amino acid sequence of SEQ ID NO:14, amino acids 20 to 307.

Claims Text - CLTX (19):
19. The method of claim 9, wherein the **phenolic acid esterase** consists of the amino acid sequence of SEQ ID NO:14, amino acids 20 to 421.

Claims Text - CLTX (21):
21. The method of claim 9, wherein the **phenolic acid esterase** is present in the edible composition at a ratio of from 0.1 to 200 U/kg dry weight of the edible composition.

Claims Text - CLTX (22):
22. The method of claim 21, wherein the **phenolic acid esterase** is present in the edible composition at a ratio of from 20 to 50 U/kg dry weight of the edible composition.

Claims Text - CLTX (28):
28. The method of claim 1, wherein the plant-derived composition is a pulping composition and wherein a **phenolic acid esterase** is added to the pulping composition at a ratio of from 0.1 to 200 U/kg dry weight.

Claims Text - CLTX (29):
29. The method of claim 28, wherein the **phenolic acid esterase** is added at a ratio of from 10 to 100 U/kg dry weight in the pulping composition.

Claims Text - CLTX (32):
32. The method of claim 28, wherein the **phenolic acid esterase is a ferulic acid esterase**.

Claims Text - CLTX (34):
34. The method of claim 33, wherein the **phenolic acid esterase** comprises an amino acid sequence as given in SEQ ID NO:18, amino acids 1-530.

Claims Text - CLTX (35):
35. The method of claim 9, wherein said **phenolic acid esterase is a feruloyl esterase** derived from Orpinomyces PC-2, Clostridium thermocellum, or a Ruminococcus species.

Claims Text - CLTX (36):
36. The method of claim 9, wherein the **phenolic acid esterase** is derived from Trichoderma, Streptomyces, Bacillus, Aureobasidium, Penicillium, Neocallimastix or Humicola.

Other Reference Publication - OREF (10):
Blum et al . (1999) "Characterization of a **Feruloyl Esterase** from the Anaerobic Fungus Orpinomyces sp. Strain PC-2"; Abstracts 99.sup.th General Meeting of the American Society for Microbiology, Chicago, IL. May 30-Jun. 3, 1999, vol. 99, pp. 430-431.

Other Reference Publication - OREF (12):
Borneman et al. (1992) "Purification and Partial Characterization of Two **Feruloyl Esterases** from the Anaerobic Fungus Neocallimastix Strain MC-2" Applied and Environmental Microbiology 58:3762-3766.

Other Reference Publication - OREF (13):
Borneman et al. (1990) "Assay for trans-**p-Coumaroyl Esterase** Using a Specific Substrate from Plant Cell Walls" Analytical Biochemistry 190:129-133.

Other Reference Publication - OREF (14):
Castanares and Wood (1992) "Purification and Characterization of a Feruloyl/**p-Coumaroyl Esterase** from Solid-State Cultures of the Aerobic Fungus Penicillium pinophilum" Biochemical Society Transactions 20:275S.

Other Reference Publication - OREF (20):
De Vries et al. (1997) "The faeA Genes from Aspergillus niger and Aspergillus tubingensis Encode **Ferulic Acid Esterases** Involved in Degradation of Complex Cell Wall Polysaccharides" Applied and Environmental Microbiology

63:4638-4644.

Other Reference Publication - OREF (21):
Faulds and Williamson (1991) "The Purification and Characterization of 4-Hydroxy-3-Methoxycinnamic (**Ferulic) Acid Esterase** from Streptomyces olivochromogenes" Journal of General Microbiology 137:2339-2345.

Other Reference Publication - OREF (36):
MacKenzie and Bilous (1988) "**Ferulic Acid Esterase** Activity from Schizophyllum commune" Applied and Environmental Microbiology 54:1170-1173.

Other Reference Publication - OREF (39):
McSweeney et al. (1998) "Butyrivibrio spp. and Other Xylanolytic Microorganisms from the Rumen have **Cinnamoyl Esterase** Activity" Anaerobe 4:57-65.

Other Reference Publication - OREF (46):
Blum et al. (2000) "**Feruloyl Esterase** Activity of the Clostridium thermocellum Cellulosome Can Be Attributed to Previously Unknown Domans of XynY and XynZ"; J. of Bacteriology 182(5):1346-1351.

US-PAT-NO: 6599745

DOCUMENT-IDENTIFIER: US 6599745 B1

TITLE: Method to isolate mutants and to clone the complementing gene

DATE-ISSUED: July 29, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
De Graaff; Leendert Hendrik	Oosterbeek		N/A	N/A	NL
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APPL-NO: 09/ 613811

DATE FILED: July 11, 2000

PARENT-CASE:

This is a divisional of application Ser. No. 08/981,729, filed Dec. 23, 1997 now U.S. Pat. No. 6,177,261, the diclosure of which is incorporated herein by reference which is a 371 PCT/NL96/00259, Jun. 24, 1996.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
EP	95201707	June 23, 1995
EP	95202346	August 30, 1995

US-CL-CURRENT: 435/471, 435/252.3 , 435/254.11 , 435/6

ABSTRACT:

The subject invention lies in the field of microorganism mutation and selection of the mutants. In particular, the invention is directed at obtaining metabolic mutants in a simple, direct and specific manner. In a preferred embodiment it is also possible to obtain desired mutants not comprising recombinant DNA, thereby facilitating incorporation thereof in products for human consumption or application, due to shorter legislative procedures. The method according to the invention involves random mutation and specific selection of the desired metabolic mutant. Knockout mutants wherein a gene associated with metabolism is absent or inactivated and mutants with increased or decreased DNA binding capacity are also claimed.

17 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

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Detailed Description Text - DETX (25):

Genes of particular interest for expressing using the expression cassette according to the invention or in combination with a nucleic acid sequence according to the invention are those encoding enzymes. Suitable genes for expressing are genes encoding xylanases, glucanases, oxidoreductases such as hexose oxidase, .alpha.-glucuronidase, lipase, **esterase, ferulic acid esterase** and proteases. These are non limiting examples of desirable expression products. A number of sequences are known in the state of the art comprising the genes mentioned and such information is readily available to the person skilled in the art and is to be considered incorporated herein. The genes can either be readily synthesized on the basis of known sequences in the literature or databases or be derived from organisms or vectors comprising them in a standard manner known per se and are considered to be knowledge readily available to the person skilled in the art not requiring further elucidation.

US-PAT-NO: 6573086

DOCUMENT-IDENTIFIER: US 6573086 B1

TITLE: Transformation system in the field of filamentous fungal hosts

DATE-ISSUED: June 3, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Emalfrab; Mark Aaron	Jupiter	FL	N/A	N/A
Burlingame; Richard Paul	Manitowoc	WI	N/A	N/A
Olson; Philip Terry	Manitowoc	WI	N/A	N/A
Sinitsyn; Arkady	Moscow	N/A	N/A	RU
Panteleimonovich	Toulouse	N/A	N/A	FR
Parriche; Martine	Quint-Fonsegrives	N/A	N/A	FR
Bousson; Jean Christophe	Manitowoc	WI	N/A	N/A
Pynnonen; Christine Marie	Houten	N/A	N/A	NL
Punt; Peter Jan	Vieuten-De Meern	N/A	N/A	NL
Van Zeijl; Cornelia Marie Johanna				

APPL-NO: 09/ 548938

DATE FILED: April 13, 2000

PARENT-CASE:

REFERENCE TO PRIOR APPLICATIONS

This is a continuation-in-part of international application PCT/NL99/00618, filed Oct. 6, 1999, which is a continuation-in-part of international application PCT/EP98/06496, filed Oct. 6, 1998.

US-CL-CURRENT: 435/254.11, 435/209 , 435/69.1

ABSTRACT:

A novel transformation system in the field of filamentous fungal hosts for expressing and secreting heterologous proteins or polypeptides is described. The invention also covers a process for producing large amounts of polypeptide or protein in an economical manner. The system comprises a transformed or transfected fungal strain of the genus Chrysosporium, more particularly of Chrysosporium lucknowense and mutants or derivatives thereof. It also covers transformants containing Chrysosporium coding sequences, as well expression-regulating sequences of Chrysosporium genes. Also provided are novel fungal enzymes and their encoding sequences and expression-regulating sequences.

25 Claims, 69 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 36

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Detailed Description Text - DETX (84):

The 100 kD protein with pI 4.5 possessed activity only toward p-nitrophenyl butyrate. It is probably an **esterase but is not a feruloyl esterase** as it had no activity against methyl ester of ferulic acid. It had neutral/alkaline pH optimum (pH 8-9) and optimal temperature of 55-60.degree. C.